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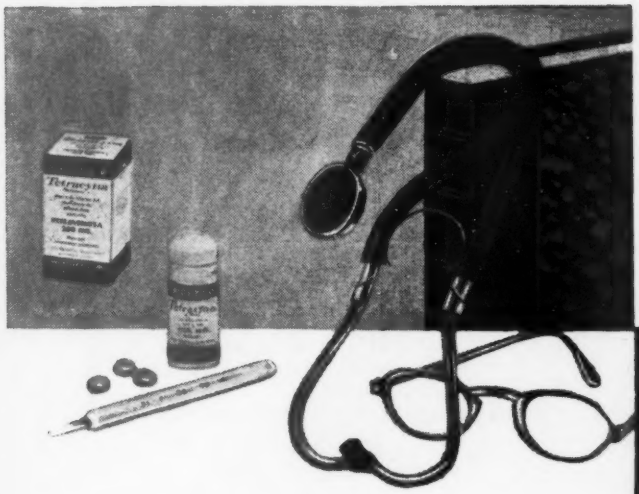

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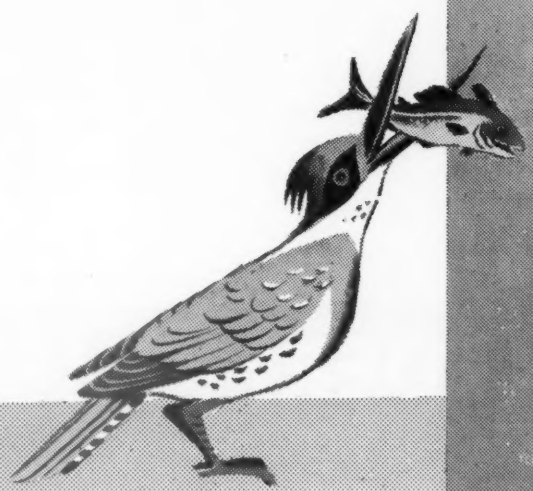

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BIOLOGICAL SIMILARITIES

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A) GENERAL CONSIDERATIONS

THE RULES of similarity are frequently applied in mechanical, hydraulic and electrodynamic constructions, in order to calculate from the physical dimensions of a model the working characteristics of the prototype. The principle of similarity is based on the assumption that all physical equations are dimensionally uniform. Any magnitude $[Q] = M^{\alpha} L^{\beta} T^{\gamma}$, where M = mass, L = length and T = time, is related to the magnitude [q] of the model by the following reduction coefficients: $M/m = \mu$, $L/l = \lambda$, $T/t = \tau$. These relations determine the value of the general reduction coefficient:

$$\chi = \mu^{\alpha} \lambda^{\beta} \tau^{\gamma}. \tag{1}$$

Thus:

$$Q = q \cdot \chi. \tag{2}$$

Let us take, for example, two spheres with the radii R and r; the ratio is $R/r = \lambda$. The surfaces of these spheres will be $S = 4\pi R^2$ and $s = 4\pi r^2$ respectively; whence the reduction coefficient is $R^2/r^2 = \lambda^2$. The same result can be obtained by applying the reduction formula (1). In this case $\alpha = 0$; $\beta = 2$, and $\gamma = 0$, since the characteristic in question is a surface. It can be noted, that if the surface of the model (s) is taken as unity, then that of the large sphere will be $S = \lambda^2$. This type of simplifying assumption will be used frequently in the present paper.

We shall consider now the case of two cubes, with edge length of $L = 5$ and $l = 2$ respectively. According to the aforementioned rules: $L/l = \lambda = 5/2 = 2.5$ and the ratio of the surfaces of the two cubes will be $S/s = \lambda^2 = (2.5)^2 = 6.25$. The ratio of the volumes is $V/v = \lambda^3 = (2.5)^3 = 15.6$. Now, let us suppose that the small cube is filled with mercury (density = 13.6) and the large one with water (density = 1.0), and we wish to compare the pressures at the bases of these cubes. The dimensions of pressure [Force/area] are $[ML^{-1} T^{-2}]$, in other words $\alpha = 1$, $\beta = -1$ and $\gamma = -2$. In consequence, the reduction coefficient is $\chi = \mu \cdot \lambda^{-1} \cdot \tau^{-2}$.

The value of $\mu = M/m$ is $\frac{L^3 \cdot D}{l^3 \cdot d}$ for $M = L^3 \cdot D$ if D = density and $m = l^3 \cdot d$. Given

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that $D/d = \delta$ then $M/m = \lambda^3 \cdot \delta$ and in this particular case $M/m = \lambda^3 \cdot \frac{1}{13.6}$, since $\delta = \frac{1}{13.6}$. The ratio $L/l = \lambda = 2.5$ is known, but no mention has been made of $\tau = T/t$. Newton established that since gravity $[g] = [LT^{-2}]$ remains constant, the reduction coefficient for gravity is $\lambda\tau^{-2} = 1$. This enables us to deduce the value of the reduction coefficient in the present case ($\tau = \lambda^{1/2}$) i.e. when replacing in $\chi = \mu \cdot \lambda^{-1} \cdot \tau^{-2}$ the above mentioned equivalences one obtains: $\chi = \lambda^3 \cdot \delta \cdot \lambda^{-1} \cdot \lambda^{-1} = \lambda\delta$ or $\frac{2.5}{13.6} = 0.184$. The same result can be obtained (0.184) by direct comparison of pressures.

The general equation $\chi = \mu^\alpha \lambda^\beta \tau^\gamma$ can be expressed as a fuction of λ and δ, since $\mu = \lambda^3\delta$ and $\tau = \lambda^{1/2}$.

$$\chi = \delta^3 \lambda^{3\alpha+\beta+\frac{\gamma}{2}} \tag{3}$$

When the density of the models being studied is the same ($\delta = 1$) then

$$\chi = \lambda^{3\alpha+\beta+\gamma/2} \tag{4}$$

known to be Newton's reduction coefficient for mechanical similarities.

In the physiological applications of this rule it is important to note that the reduction coefficient for mass (M) and weight ($P = M \cdot g$) are the same, since gravity g is constant. Thus $P/p = M/m = \lambda^3 \cdot \delta$. If one accepts that the density of similar models is practically constant, then $P/p = \lambda^3$, and if in the reference model $p = 1.0$, it follows that $\lambda = P^{1/3}$. In this case P is numerically —but not dimensionally— equal to the weight, since p is considered equal to unity.

As an example of the importance of the time factor (T), let us apply the rule of similarity to a mathematical pendulum. The problem consists in the calculation of the ratio (F/f) between the frequencies of two ideal pendulums which differ only in length. In this case we have $M/m = \mu = 1.0$, in other words $\alpha = 0$; as $L/l = \lambda$ it follows that $\beta = 1.0$. As frequency is dimensionally $[T^{-1}]$ the reduction coefficient has $\alpha = 0$, $\beta = 0$ and $\gamma = -1$. Therefore $\chi = \lambda^{-1/2}$ and F/f will be $\lambda^{-1/2}$.

Another way of approach is to assume that $F = \frac{1}{2\pi\sqrt{L/g}}$ hence

$$\frac{F}{f} = \frac{2\pi\sqrt{l/g}}{2\pi\sqrt{L/g}} = \sqrt{l/L} = \lambda^{-1/2}.$$

However, the expression $\lambda\tau^{-2} = 1.0$ which has been applied in the above examples is not valid in electrodynamics, since the electrons are practically not subjected to the action of gravity owing to the smallness of their mass. In this case a new relation between λ and τ holds, namely $\lambda\tau^{-1} = 1$. This equation is derived from the constant factor $1/\sqrt{\varepsilon \cdot \mu} = v$, where ε = dielectric constant, μ = magnetic permeability, and v the speed of light. This latter relationship is used to obtain dimensional uniformity in all electrodynamic equations. The reduction formula for electrodynamic models, assuming that the constituents do not change from one to another, will be:

$$\lambda^{3\alpha+\beta+\gamma}. \tag{5}$$

This formula can be applied, for instance, to compare two similar electric pendulums. Let us consider their characteristic frequencies. An electric pendulum is made up of a condenser (C) and an inductance (H). The frequencies of the two

pendulums are given by $F = \frac{1}{2\pi\sqrt{H \cdot C}}$. The dimensions of frequency is $[T^{-1}]$ and if we apply the reduction formula (5) then $F/f = \lambda^{-1}$ is obtaind. This same relation can be calculated by direct comparison, using the formula for the frequency of each pendulum: $\frac{F}{f} = \frac{2\pi\sqrt{h \cdot c}}{2\pi\sqrt{H \cdot C}}$ Since the magnetic induction $H = \frac{4\pi\mu NA}{l}$ is dimensionally equal to $[\mu L]$ while $h = [\mu l]$ and the capacity $C = \frac{\varepsilon A}{4\pi d}$ is dimensionally equal to $[\varepsilon L]$ and $c = [\varepsilon \cdot l]$ then $\frac{F}{f} = \sqrt{\frac{\mu l \cdot \varepsilon l}{\mu L \cdot \varepsilon L}} = \frac{l}{L} = \lambda^{-1}$.

In general, the application of the electrodynamic and mechanical reduction formulas solves many problems in these fields, without previous knowledge of the empirical equation for each particular case.

B) Biological applications

Living beings are governed by the laws of physics, and therefore it is possible to apply in a certain way the rules of similarity, in order to compare their functional characteristics. It is also obvious, that neither of the rules of similarity, discussed in the first part of this paper, can be applied strictly to living beings, for these are governed in part by the laws of mechanics and partly by those of electrodynamics. The metabolic processes entail a flow of electrons and protons, from hydrogen to oxygen, and therefore are fundamentally electrodynamic in nature.

With the above considerations in mind, and accepting the universal nature of the laws of classical mechanics and electrodynamics, it is possible to postulate:

1. that all living beings are governed simultaneously by the rules of mechanical and electrodynamic similarity;
2. that the density and the nature of the substances of which they are made up are constant; and
3. that "biological similarities" will have a reduction formula χ^x, and that the value of the exponent x will vary between the values of the exponents of the mechanical and electrodynamic similarities.

As a consequence of these postulates, the reduction coefficient of the biological similarities will be the following:

$$\lambda^{3\alpha+\beta+\gamma'} \tag{6}$$

where the value of γ' for each biological similarity varies between $\gamma/^2$ and γ.

Considering that the density of all living beings is approximately constant, a reduction coefficient as a function of weight (P) can be obtained, by utilizing the relation $P = \lambda^3$ and using as a model of reference those of unit weight (1 kg). Every biological function (A) will thus be related to the corresponding model (a) by the formula:

$$A = a P^{\frac{1}{3}(3\alpha+\beta+\gamma')}. \tag{7}$$

This equation is equivalent to the heterogenic formula of Huxley: $Y = aX^b$, where Y is the function under study, X the body weight, a and b two parameters. This formula has been extensively used by many authors

TABLE I

Illustrating the calculation of the exponents of reduction coefficients in relation to weight (P) for three similarities (mechanical M, electrodynamic E and biological B).

Quantity	Dimensions			Similarities		
	M	L	T	M	E	B
	α	β	γ			
Acceleration	0	1	−2	0.00	−0.33	−0.29
Action (dynamics)	1	2	−1	1.50	1.33	1.36
Area	0	2	0	0.66	0.66	0.66
Density	1	−3	0	0.00	0.00	0.00
Diffusivity	0	2	−1	0.50	0.33	0.36
Elastic modulus	1	−1	−2	0.33	0.00	0.05
Energy and work	1	2	−2	1.33	1.00	1.05
Flow	0	3	−1	0.83	0.66	0.69
Force	1	1	−2	1.00	0.66	0.72
Frequency	0	0	−1	−0.16	−0.33	−0.31
Length	0	1	0	0.33	0.33	0.33
Moment of force	1	2	−2	1.33	1.00	1.05
Moment of inertia	1	2	0	1.66	1.66	1.66
Momentum and impulse	1	1	−1	1.16	1.00	1.02
Period	0	0	1	0.16	0.33	0.31
Power	1	2	−3	1.16	0.66	0.73
Pressure and stress	1	−1	−2	0.33	0.00	0.05
Peripheral resistance	1	−4	−1	−0.50	−0.66	−0.64
Speed or velocity	0	1	−1	0.16	0.00	0.02
Surface tension	1	0	−2	0.66	0.33	0.38
Viscosity	1	−1	−1	0.50	0.33	0.36
Volume	0	3	0	1.00	1.00	1.00

to describe the relationship between various functions and body weight. The exponent b of this formula is equal to $1/3\,(3\alpha + \beta + \gamma')$ and the parameter a corresponds to the value of the functions in the reference model. If in equation (7) the value of γ' is replaced by $0.93\,\gamma$ we obtain a reduction formula of biological similarity, which in practice is valid for a great many functions in mammals:

$$A = a\,P^{1/3\,(3\alpha+\beta+0.93\gamma)} \qquad (8)$$

Table I shows examples of the applications of the theory of si-

milarity to various physical magnitudes, all of which are correlated with weight (P). For instance, if power is expressed dimensionally as $[ML^2 T^{-3}]$ one obtains the following reduction formulas for:

a) Mechanical Similarity; since $\alpha = 1$; $\beta = 2$ and $\gamma = -3$ we have $P^{1/3(3+2-3/2)} = P^{1.16}$.

b) Electrodynamic Similarity; we have $P^{1/3(3+2-3)} = P^{0.66}$.

c) Biological Similarity; accordingly $P^{1/3(3+2-2.79)} = P^{0.736}$.

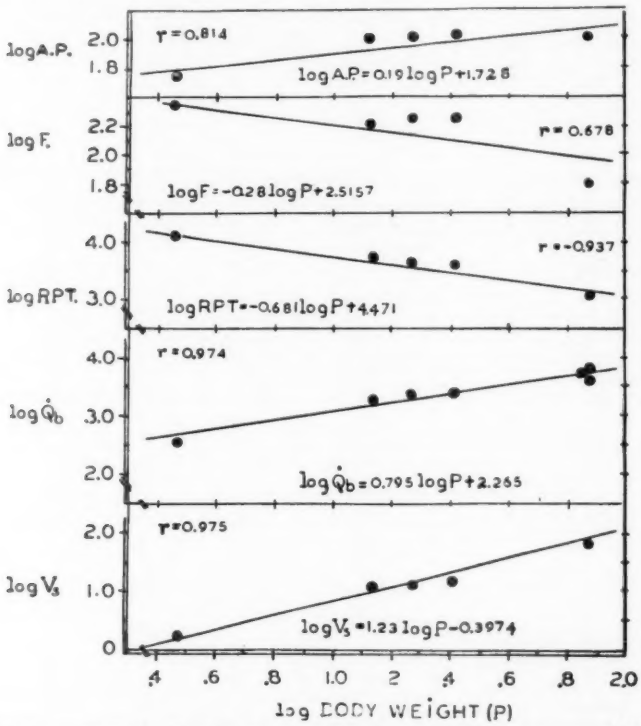
log A.P.
r = 0.814
log A.P = 0.19 log P + 1.728
log F
r = 0.678
log F = -0.28 log P + 2.5157
log RPT
r = -0.937
log RPT = -0.68 log P + 4.471
log $\dot{Q}_b$
r = 0.974
log $\dot{Q}_b$ = 0.795 log P + 2.265
log V_s
r = 0.975
log V_s = 1.23 log P - 0.3974
log BODY WEIGHT (P)

FIG. 1. — *Graphs relating the logarithms of arterial pressure (AP), heart frequency (F), total peripheral resistance (RPT), minute-volume (Q_b) and systolic volume (V_s), to the logarithm of body weight (P). The correlation coefficients (r) and the corresponding equations are also shown in this figure. For references see Table II.*

C) SPECIAL BIOLOGICAL APPLICATIONS

The experimental data at present available to verify the theory of biological similarity are limited, for only in a few cases have statistical treatments of the results been made. Many authors do not give details of the weights of the animals used; in other instances the weight range is very small, and in several cases the experimental methods employed in different laboratories are not the same. Nevertheless, with the

TABLE II

Exponents of body weight (P) of different functions of the cardiovascular system.

Functions	Units	Theoretical values			Experimental equations	S. E. "b" *	References
		M	E	B			
Viscosity (defibrinated blood)	In relation with water	0.50	0.33	0.36	$3.78\ P^{0.097}$	0.0434	23
Total blood volume	ml	1.00	1.00	1.00	$0.055\ P^{0.99}$	—	1 6
Systolic volume	ml	1.00	1.00	1.00	$0.40\ P^{1.232}$	0.161	22 27 29
Minute-volume	ml/min	0.83	0.66	0.69	$184\ P^{0.795}$	0.0727	22 27 29
Arterial pressure	mmHg	0.33	0.00	0.05	$110.7\ P^{0.032}$	0.0175	24
Total peripheral resistance	*dynes/sec.* cm^5	— 0.50	— 0.66	— 0.64	$29580\ P^{-0.68}$	0.147	22 27 29
Frequency of the heart	f/min	— 0.16	— 0.33	— 0.31	$328\ P^{-0.28}$	0.175	22 27 29
Duration of one heart-beat	hours	0.16	0.33	0.31	$0.0000119\ P^{0.27}$	—	1

* S.E."b", Standard Error of the Exponent "b".

data at hand it is possible to study several functions in relation to body size.

In the following section we will present the results of the applications of the theory of biological similarity to several physiological functions.

1. *Cardiovascular system.* — Table II shows the equations corresponding to various functions of the cardiovascular apparatus (see Fig 1). Some of the figures presented are only tentative values, for they have been collected from various sources and the experimental methods used are not always strictly comparable. This is specially true for cardiac output determinations, where technique and anaesthesia vary considerably.

TABLE III

Equations of various respiratory functions of mammals of different body size.

Function	Theoretical values			Experimental equations	S. E. "b"	Units	References
	M	E	B				
Minute-volume	0.83	0.66	0.69	$7.31\ P^{0.669}$	0.0177	ml/min	7
				$1.99\ P^{0.756}$	0.0363	ml/min	9
Tidal air	1.00	1.00	1.00	$0.0116\ P^{0.97}$	0.0681	ml	7
				$0.006\ P^{1.02}$	0.0821	ml	9
Respiratory frequency	— 0.16	— 0.33	— 0.31	$547\ P^{-0.298}$	0.0536	min	7
				$379\ P^{-0.29}$	0.0436	min	9

In the case of the exponent of heart rate in relation to body weight the theoretical value lies between the figures given by Clark, as cited by Brody ([6]) (p. 932) and those of von Bertalanffy ([2]) (p. 256).

It was also possible to predict some morphologic characteristics of the cardiovascular system in relation to body weight (P). Thus, the lengths of arteries, capillaries and veins should be proportional to $P^{0.33}$ and the corresponding cross-sections to $P^{0.66}$. For instance, several authors have found [for references see Günther ([11])] the cross-section of the aorta be $A = a\ P^{0.70}$.

The total volume of the circulatory system ($V = L \cdot A$) is a function of $P^{1.0}$, since $P^{0.33} \cdot P^{0.66} = P^{1.0}$. This result is in close agreement with experimental facts (see Table II), for it has been shown for different species [Brody ([6]), p. 593] that the total blood volume is proportional to $P^{0.987}$. For this reason a constant relation must exist between the systolic volume and the total volume of the arterial system, since both vary as $P^{1.0}$.

The wall tension (W) of the blood vessels varies according to Laplace's formula $W = p \cdot r$, where p = pressure and r = radius. The tension in any particular vessel increases in relation to body weight (P) in accordance with $W = P^{0.05} \cdot P^{0.33} = P^{0.38}$.

The velocity of the blood (v) in a certain territory is practically independant of body weight, for it varies with $P^{0.02}$, since:

$$v = \frac{L}{T} = \frac{P^{0.33}}{P^{0.31}} = P^{0.02}.$$

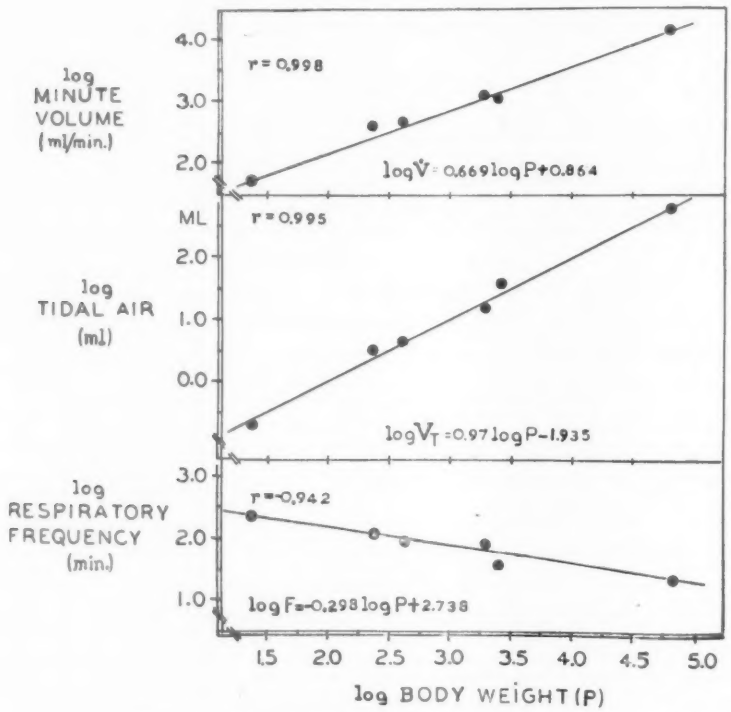

FIG. 2. — *Respiratory functions in relation to body weight* ([7]).

2. *Respiratory system.* — Two excellent papers ([7], [9]) have been published in which the functional characteristics of the respiratory system of mammals were studied in relation to body weight. The results of these measurements are presented in Table III and Fig 2. The agreement between the observed values and those predicted by the rules of biological similarity are noteworthy. Von Bertalanffy ([2]) (p. 259) has described almost the same relationship ($P^{-1/3}$) for the respiratory frequency of birds as well as that of mammals.

The respiratory dead space (V_D) does not appear in Table III; however, according to our submission, the "respiratory dead space" should vary with $P^{1.0}$. Now, as tidal volume (V_T) is also a function of $P^{1.0}$, the

relation between these two $\frac{V_D}{V_T}$ should be a constant. It is interesting to note that observed relation ([12]), as determined experimentally, actually is constant; namely $\frac{V_D}{V_T} = 1/3$.

The speed of air in the respiratory tract can be calculated from the minute-volume and the respiratory cross-sections (Flow/area). It has been shown ([10]) that the velocity of air $\frac{P^{0.75}}{P^{0.70}} = P^{0.05}$ is practically independant of body weight. Again, the agreement between the theoretic predictions ($P^{0.03}$) and the observed facts is close enough. In the rat the calculated ([10]) inspiratory air speed is 2.05 m/sec and in man 2.95 m/sec; while expiratory speed is 2.65 m/sec in the rat and 2.8 m/sec in man.

TABLE IV

Clearance (ml/h) of different substances expressed in relation to body weight (after Adolph) [1].

Clearance of	Experimental equations
Urea	$1.59\ P^{0.72}$
Inulin	$1.74\ P^{0.77}$
Creatinine	$4.2\ P^{0.69}$
Diodrast	$2.14\ P^{0.89}$
Hippurate	$5.4\ P^{0.80}$

3. *Renal function.* — According to the concept of biological similarity, renal clearance values should vary with $P^{0.69}$. In Table IV the experimentally found clearances are expressed in relation to body weight ([1]). The theoretic range varies between $P^{0.66}$ and $P^{0.83}$. Only the diotrast clearance has a value outside this range.

Urinary minute-volume has also been studied ([1]), and the following equation obtained; $U = 0.0064\ P^{0.82}$. The urinary excretion of catabolites (g/h) can be expressed as a function of body weight and the following relations were obtained; for total nitrogen $N_T = 0.000074\ P^{0.735}$; for endogenous nitrogen $N_E = 0.000042\ P^{0.72}$; for sulphur excretion $S = 0.0000071\ P^{0.74}$. These exponents agree well with those predicted theoretically ($P^{0.69}$). There is however one exception, namely creatinine elimination $Nc = 0.0000091\ P^{0.90}$. This might be explained if one considers that creatinine excretion is a function of muscular metabolism, and muscle mass varies in proportion to body weight ($P^{1.0}$).

4. *Basal metabolism.* — In all living organisms oxygen consumption and carbon dioxide production are basic physiological phenomena. Other functions such as circulation, respiration and excretion depend on metabolic intensity. According to biological similarity, the metabolism (power) should vary with $P^{0.73}$ if $P^{0.66}$ and $P^{1.16}$ are the theoretic limits.

Table V shows the experimentally determined equations for different animals. It is interesting to note, that metabolism varies with the same power of body weight ($P^{0.73}$) both in homeotherms and in poikilotherms; the value of the parameter a is however 37.7 times smaller in the latter group.

TABLE V

Basal metabolism (Cal/day) of different animals.
The theoretical value, according to biological similarity, is $P^{0.736}$

Metabolism of		Experimental equations	References
Mammals		$70.5\ P^{0.734}$	6
Birds		$89\ P^{0.64}$	6
Hibernating animals	a) summer	$60.9\ P^{0.62}$	16
	b) winter	$1.97\ P^{0.94}$	
Amphibia. Reptiles		$1.87\ P^{0.728}$	16

In hibernating animals ([16]) there is a marked change of metabolism from summer ($M_s = 60.9\ P^{0.62}$) to winter ($M_w = 1.97\ P^{0.94}$). Not only does the exponent (b) of weight change but also the parameter a, the value of which decreases markedly in winter, reaching that of poikilotherms. Kayser ([16]) attributes the change of the weight exponent to the quantity of reserve-fat stored for the period of hibernation.

An extensive study of the metabolism of many living organisms has been carried out recently by Zeuthen ([30, 31]). This author has collected metabolic data of organisms ranging from bacteria to large mammals, which cover a weight range of 10^{18} and a metabolic range of 10^{15}. The experimental results described by Zeuthen ([31]) show that the exponent of weight varies between $P^{0.66}$ and $P^{1.0}$. Moreover, von Bertalanffy ([2]) has established independently the same range for the exponents of body weight. Their values are within those predicted by the present theory.

The exponent of metabolism ($P^{0.73}$) shows clearly that electrodynamic factors predominate in animal metabolism, for they are close to the value $P^{0.66}$ of electrodynamic similarity. This is not an unexpected result, since metabolism is essentially a transport of electrons and protons (hydrogen ions), from the substrate (— 0.42 V) to oxygen (+ 0.8 V). It is well

known, that a molecule of oxygen can capture 4 electrons and 4 protons to yield water ($O_2 + 4\,H^+ + 4\,e^- = 2\,H_2O$). Therefore, the equivalence between oxygen consumption, heat production and amperage can be established according to the laws of energetic equivalence. For example, a man consuming 0.25 L of oxygen per minute produces 1·2 Cal/min, which is equivalent to an electric current of 70 Amperes or 84 Watts, since the potential difference in the cells is only 1.2 Volts.

If one expresses mammalian metabolism per unit weight, the following theoretical relation is obtained $\frac{\text{Metabolism}}{\text{Weight}} = \frac{P^{0.73}}{P^{1.0}} = P^{-0.27}$. The experimental values obtained by measuring the oxygen consumption "in vitro" of tissues of animals of different size ([3], [18], [28]) accord with the aforementioned expression. These results warrant the conclusion that isolated tissues show no alteration in respect of the metabolic intensity of the tissues of the whole animal. However, several authors ([3], [5], [20]) have been unable to confirm these results. For instance, von Bertalanffy et al. [4]) have shown that the decrease of oxygen consumption per unit weight is not very marked, and varies only as a function of $P^{-0.10}$. Elliot ([8]) has found that this latter relation holds for brain metabolism "in vitro". Apparently, when considering all the experimental results, the diaphragm is one of the few tissues in which oxygen consumption per unit weight is a function of $P^{-0.26}$ and accords with then mean theoretic value ([3], [5]). Finally, it is interesting to note, that the concentration of a respiratory enzyme ([25]), cytochrome c, is also related to body weight ($P^{-0.278}$).

5. *Nervous system.* — We have been unable to find data on the various functions of the nervous system in relation to body size. Nevertheless, we should like to discuss some theoretic considerations which may be of interest. The length of homologous nerves increases with $P^{0.33}$ and their cross-sections with $P^{0.66}$. According to von Hoesslin ([14]) (p. 363), the total number of fibers reaching an organ should be proportional to $P^{0.66}$. The velocity of nerve impulse transmission should be almost constant in species with different body weight, for the predicated value is $P^{0.02}$.

Nervous functions which depend on time (duration of the latency period, of action potential, of the refractory periods, etc) should vary with $P^{0.31}$ (limits: $P^{.016}$ and $P^{0.33}$). On the contrary, all frequencies must obey the following relation: $P^{-0.31}$.

6. *Muscular system.* — For muscular functions we can deduce similar relations. Thus, utilization time, chronaxie, the duration of a contraction, the refractory period, etc, should vary with $P^{0.31}$; and all frequencies with $P^{-0.31}$.

The length of homologous muscles is proportional to $P^{0.33}$; their cross-sections to $P^{0.66}$; the muscle masses to $P^{1.0}$; and muscle force, for instance, should vary with $P^{0.72}$ (see Table I).

7. *Skeleton.* — The length of homologous bones varies with $P^{0.33}$ and their cross-sections with $P^{0.66}$. The stress sustained by a section of bone equals: $\frac{\text{Force}}{\text{Area}} = \frac{P^{0.72}}{P^{0.66}} = P^{0.06}$. This indicates that the stress to which

bones are submitted is nearly constant for a given bone, and only increases very slightly with body weight.

Resistance to torque ($F \times d$), where F = force and d = distance, varies with $P^{0.72} \cdot P^{0.33} = P^{1.05}$. In other words, torque increases in proportion to body weight, a relation first described by von Hoesslin [14]. The dimensional equations for stress and torque are shown in Table I.

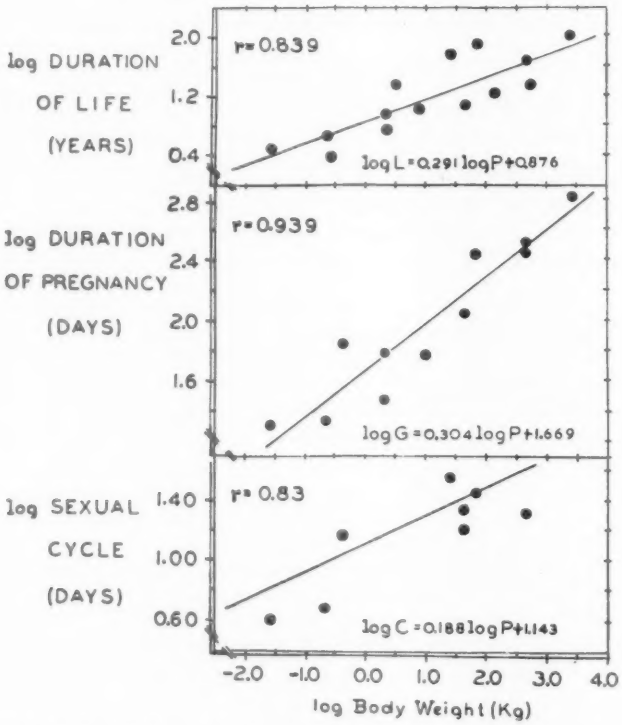

FIG. 3. — *Biological periods in relation to body weight. For references see Table VI.*

8. *Time factor in Biology.* — As discussed previously, the only difference between the three similarities (mechanical, electrodynamic and biological) lies in the differences of the exponents of time (γ). For biological similarity in mammals we have suggested as the most widely applicable formula the following: $A = a\, P^{1/3(3\alpha+\beta+0.93\gamma)}$. From this equation it can be deduced that frequencies vary with $P^{-0.31}$ and periods with $P^{0.31}$. In Table VI the experimental results obtained in homeotherms for the duration of certain physiological functions are presented. It can be seen that the same biological process is carried out faster in a small animal than in a larger one. In living beings, all cyclic processes are repeated according to a period dependent on body weight (Fig 3). In a figurative manner one could say, that each organism has its own pacemarker or "biological clock", which determines the rhythm of each

function (heart rate, respiratory rate, durations of sexual cycle, pregnancy, growth and life).

It is interesting to note, that von Hoesslin [15] has measured the time necessary for an organism to reach the adult weight and he found that in certain animals and plants this is a function of $P^{0.33}$.

On the basis of Brody's [6] data on growth (his Table N° 16.1,

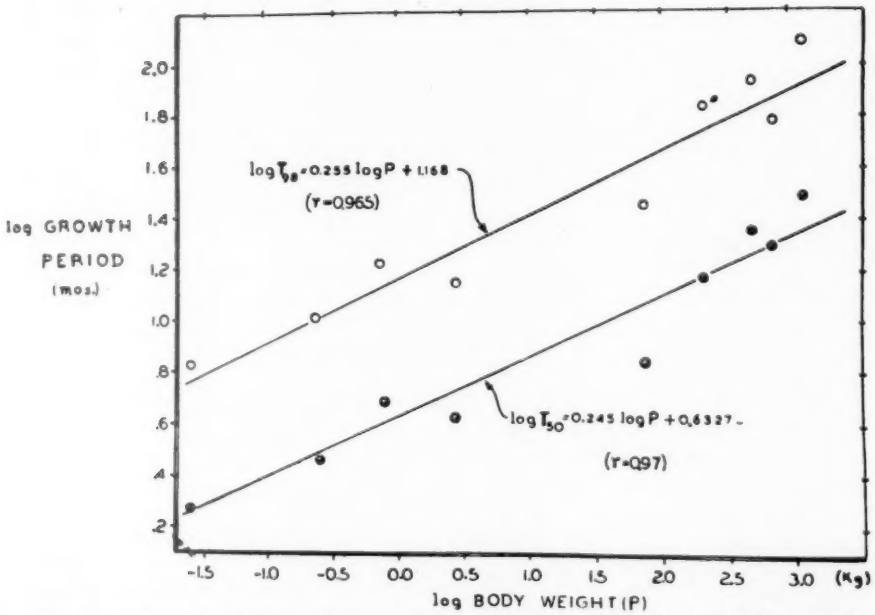

FIG. 4. — *Growth periods in relation to body weight. T_{98} is the time required to reach 98 % of mature weight, and T_{50} the time necessary to reach 50 % of mature weight* [6].

p. 567) it is possible to calculate (Fig 4) that the half-time of growth, i. e. the time necessary to reach 50 % of adult weight, is equal to 4.29 $P^{0.245}$, and the 98 % of mature weight can be expressed as 14.74 $P^{0.255}$.

Summarizing it can be stated that "homologous periods" corresponds to the time necessary for carrying out a certain function. Fig 5 shows the duration of one heart cycle as a function of body weight; the smaller the animal, the shorter is the duration of one cardiac cycle, or of any other periodic phenomenon.

9. *Biological constants.* — It is possible to compare two functions which have the same exponent (b) in Huxley's equations ($Y = aX^b$) and only differ in the value of the parameter (a). The quotient obtained is a number representing an absolute "biological constant", which is independant of body weight. For example, in homeotherms the relation between:

$$\frac{\text{Duration of one respiratory cycle}}{\text{Duration of one cardiac cycle}} = \frac{0.0000470\ P^{0.28}}{0.0000119\ P^{0.27}} = 4.0$$

which indicates that for one respiration we have four heart beats.

In a similar manner, a constant relation between the duration of pregnancy and the duration of life can be obtained (see Table VI).

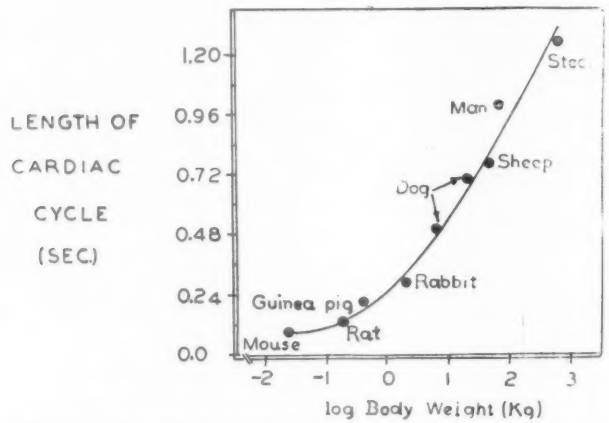

FIG. 5. — *Length of heart cycle in relation to the logarithm of body weight* ([5]).

Other important ratios are those between heart rate and metabolism per unit weight (ml 0_2/Kg/min), leading to a constant of 21.0, and the relation between respiratory frequency and metabolism per unit weight, with a constant of 5.4.

Furthermore, it can be calculated that the total number of heart beats or respiratory cycles carried out during a life-time are approximately constant, if the weight of the adult animals is used as a reference point. The duration of life (years) is a function of $P^{0.31}$, and all frequencies vary according to P^{-031}. The product of $P^{0.31} \cdot P^{-0.31} = P^{0.0} = 1.0$.

The same relation is applicable to metabolism per unit weight ($P^{-0.266}$). Thus, in the course of life ($P^{0.30}$) the total number of calories per Kg body weight will be approximately constant ($P^{0.034}$). This fact has been established in 1908 by Rubner and mentioned by Brody ([6]), who calculated that 1 Kg of any animal during its life produces about 200.000 Calories. Man, however, is an exception, for he consumes 800.000 Calorie/Kg, which might by due to his abnormally long growth period (Brody ([6]), p. 679).

D) DISCUSSION

It must be made clear that biological similarity, understood in the sense of the so called physical similarities —of form and function— does

TABLE VI

Experimental equations for different biological periods. The theoretical value according to biological similarity is $P^{0.31}$

Duration of	Units	Equations	S. E. "b"	References
Heart-beat	hours	$0.0000119\ P^{0.27}$	—	1
Respiratory cycle	hours	$0.0000470\ P^{0.28}$	—	1
Intestinal contraction	hours	$0.0000930\ P^{0.31}$	—	1
Growth (50 % mature weight)	mos.	$4.29\ P^{0.245}$	0.0283	6
Growth (98 % mature weight)	mos.	$14.74\ P^{0.255}$	0.02646	6
Sexual cycle	days	$13.9\ P^{0.188}$	0.0563	26
Pregnancy	days	$46.7\ P^{0.304}$	0.0374	26
Life	years	$7.516\ P^{0.291}$	0.0544	12

not and cannot exist, for the growth of living beings and their organs follows Huxley's heterogonic formula ($Y = aX^b$), or von Bertalanffy's [2] allometric one, and not a linear equation of proportional growth ($Y = aX$).

As Brody [6] (p. 580) pointed out "Large and small animals are not similar geometrically but rather tend to be physiologically, i. e. homeostatically, similar. The organism changes geometrically so as to remain the same physiologically".

The biological applications of the heterogonic formula have been extensively discussed by Brody [6], Bertalanffy [2], Günther [11] and Adolph [1].

In this paper physical similarities (mechanical and electrodynamic) have been useful in establishing the limits within which the biological functions will be found. Biological similarity should be considered as a statistical approximation, which reflects the adaptation of various functions to the conditions of internal and external environments. Furthermore, the value of $0.93\ \gamma$ for the time exponent of biological similarity was established empirically for mammals, because it allowed an adequate description of many of their functions. It must be pointed out that there are also functions which deviate more or less from this empirically established value. However, no variations have been found to fall outside

the limits 0.5 γ and 1.0 γ corresponding to the physical similarities (mechanical and electrodynamic).

The agreement between the experimental facts and the present theory for the functions of living beings, ranging from micro-organisms to the largest mammals, is satisfactory. Not only is an adequate description of various functions obtained, but the application of the proposed similarity rule permits a rational approach to biological processes, and clearly establishes that any biological function must vary with body weight in a manner which can be predicted "a priori". The concept of biological similarity is a useful working hypothesis in studying functions whose relation to body weight has not been established up to now.

In accordance with the above mentioned considerations, we shall term "biological similarity" the conditions which determine the placing of biological functions within the limits given by the physical similarities.

It is interesting to note that already in 1888 von Hoesslin ([14]) tried to apply the principles of mechanical similarity to living beings, and that in 1927 Lambert and Teissier ([21]) proposed a theory of "biological similitude", which happens to be the same as electrodynamic similarity discussed in this paper. Nevertheless, the isolated application of the rules of electrodynamic similarity to biological functions does not give an adequate description of the relations of various functions to body weight. Recently Kleiber ([19]) has suggested, that "the principle of similarity offers a good basis for expressing relations of anatomical and physiological variables to body size by equations whose terms have a biological meaning". In the present paper only the physiological aspects of the similarity problem are discussed.

Summary

The rules of mechanical and electrodynamic similarity cannot be applied directly to living beings. A tentative approach to a "biological similarity" is made, based on these two principles of similarity.

Biological functions are shown to vary in a predictable manner in relation to body weight in close agreement with this theory of biological similarity. It is possible to find a general equation $A = aP^{1/3(3\alpha+\beta+c\gamma)}$ where c can vary between 0.5 and 1.0. These values of c are the limits given by mechanical and electrodynamic similarities.

Numerous quantitative examples of the variation of physiological functions with body weight are given which can be analyzed according to this theory.

It is pointed out, that the rule of biological similarity discussed in this paper is applied only to functional problems of living organisms and not to their morphologic characteristics.

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We also wish to acknowledge our debt to Miss Gabriela Díaz for her assistance in the statistical calculations.

REFERENCES

(1) ADOLPH, E. F.: Quantitative relations in the physiological constitution of mammals. *Science*, 1949, *109*, 579.

(2) BERTALANFFY, L. v.: *Theoretische Biologie*. Bd. II. A. Francke A. G. Verlag, Bern, 1951.

(3) BERTALANFFY, L. v., PIROZYNSKI, W. J.: Tissue respiration and body size. *Science*, 1951, *113*, 599; 1951, *114*, 306.

(4) BERTALANFFY, L. v., ESTWICK, R. R.: Tissue respiration of musculature in relation to body size. *Amer. J. Physiol.*, 1953, *173*, 58.

(5) BERTALANFFY, L. v., PIROZYNSKI, W. J.: Tissue respiration, growth, and basal metabolism. *Biol. Bull.*, 1953, *105*, 240.

(6) BRODY, S.: *Bioenergetics and Growth*. Reinhold. Publ., New York, 1945.

(7) BUCHER, K.: Vergleichende Charakterisierung der Lungenatmung einiger Säuger. *Helv. Physiol. Acta.*, 1949, *7*, 470.

(8) ELLIOT, K. A. C.: Metabolism of brain tissue slices and suspensions from various mammals. *J. Neurophysiol.*, 1948, *11*, 473.

(9) GUYTON, A. C.: Measurement of the respiratory volumes of laboratory animals. *Amer. J. Physiol.*, 1947, *150*, 70.

(10) GUYTON, A. C.: Analysis of respiratory patterns in laboratory animals. *Amer. J. Physiol.*, 1947, *150*, 78.

(11) GÜNTHER, B.: Peso del cuerpo y metabolismo. *Bol. Soc. Biol. Concepción* (Chile), 1944, *18*, 45.

(12) HATCH, T., COOK, K., PALM, P. E.: Respiratory dead space. *J. appl. Physiol.*, 1953, *5*, 341.

(13) HEILBRUNN, L. V.: *An Outline of General Physiology*. (3rd Edit.). W. B. Saunders, Philadelphia, 1952, p. 685.

(14) HOESSLIN, H. v.: Ueber die Ursache der scheinbaren Abhängigkeit des Umsatzes von der Grösse der Körperoberfläche. Du Bois-Reymond's. *Arch. Physiol.*, 1888, p. 323.

(15) HOESSLIN, H. v.: Die Wachstumskurve. *Z. Biol.*, 1934, *95*, 477.

(16) KAYSER, CH.: Le problème de la loi des tailles et de la loi des surfaces tel qu'il apparait dans l'étude de la calorification des batraciens et reptiles et des mammifères hibernants. *Arch. Sc. Physiol.*, 1950, *4*, 361.

(17) KAYSER, CH.: La loi de surfaces. *Rev. Scient.*, 1951, *89*, 267.

(18) KLEIBER, M.: Body size and metabolism of liver slices in vitro. *Proc. Soc. Exper. Biol. & Med.*, 1941, *48*, 419.

(19) KLEIBER, M.: Physiological meaning of regression equations. *J. appl. Physiol.*, 1950, *2*, 417.

(20) KREBS, H. A.: Body size and tissue respiration. *Biochim. Biophys. Acta*, 1950, *4*, 429 (quoted by Bertalanffy)[4].

(21) LAMBERT, R., TEISSIER, G.: Theorie de la similitude biologique. *Ann. Physiol.*, 1927, *3*, 212.

(22) MEIER, R., TRIPOD, J., WIRZ, E.: Fortlaufende Bestimmung des Schlagvolumes mit Hilfe des Fickschen Prinzipes. Beeinflussung der Herzleistung am Kaninchen durch Coramin und Adrenalin. *Helv. Physiol. Acta*, 1949, *7*, 210.

(23) NEUSCHLOSZ, S. M.: *Die Viscosität des Blutes. Handb. Norm. Pathol. Physiol.* Springer, Berlin, 1928. Bd. VI/1, p. 624.

(24) PROSSER, C. L. (EDIT.): *Comparative Animal Physiology*. W. B. Saunders Co., Philadelphia, 1950, p. 538.

(25) ROSENTHAL, O., DRABKIN, D. L.: The cytochrome content of normal and neoplasic mammalian epithelium, and its correlation with body mass. *J. Biol. Chem.*, 1943, *150*, 131.

(26) SELYE, H.: Textbook of Endocrinology. *Acta Endocrinologica*, Montreal, 1948, p. 803; 816.

(27) TAYLOR, H. L., TIEDE, K.: A comparison of the estimation of the basal cardiac output from linear formula and the "cardiac index". *J. clin. Invest.*, 1952, *31*, 209.

(28) WEYMOUTH, F. W., FIELD, II, J., KLEIBER, M.: Relationship between body size and metabolism. *Proc. Soc. Biol. & Med.*, 1942, *49*, 367.

(29) WIGGERS, H.: Cardiac output and total peripheral resistance measurements in experimental dogs. *Amer. J. Physiol.*, 1943/44, *140*, 519.

(30) ZEUTHEN, E.: Body size and metabolic rate in the animal kingdom with special regard to the marine micro-fauna. *Trav. Lab. Carlsberg, Ser. Chim.*, 1947, *26*, 17.

(31) ZEUTHEN, E.: Oxygen uptake as related to body size in organism. *Quart. Rev. Biol.*, 1953, *28*, 1.

ACTION OF HISTOTOXIC ANOXIA ON THE SALIVARY AND CARDIORESPIRATORY CONDITIONAL REFLEXES

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INTRODUCTION

IT HAS BEEN SHOWN ([3], [13]) that the cardiac and respiratory reactions produced by histotoxic anoxia (injection of 0.2 mg/kg of cyanide) are easily conditioned.

In the experiments of conditioning of histotoxic anoxia, a tendency intensity, it produces a cortical inhibition. Gantt ([5]) found that an oxygen defficiency equivalent to a height of 8 000 meters, diminished an alimentary salivary conditional reflex to 60 % of its initial values, producing also a disinhibition of differentiation. Malmejac & Plane ([7], [6], [9], [10]) found that at 3 000 meters (15 % oxygen) a considerable raise of a salivary conditional reflex of defence to acids was obtained, but at 6 000 to 8 000 m (9.5 % oxygen) this reflex diminished or disappeared; both effects, the raise at 3 000 m and the inhibition at 6 000-8 000 m, decreased in succesive experiments, and after some repetitions the effects of anoxemic anoxia were practically of no account.

In the experiments of conditioning of histotoxic anoxia, a tendency to remain lying down was observed in the dogs, completely inactive and drowsy, indicating that the dose of cyanide used produced a cortical inhibition.

From the stated facts two problems aroused: the first, to prove with an objective and quantitative indicator (a salivary conditional reflex) if the dose of 0.2 mg/kg of cyanide really produced a cortical inhibition; the second: to find out how the cardiorespiratory reactions produced by cyanide were conditioned in conditions of cortical inhibition (if the first was proved).

A comparative study of the effects of histotoxic anoxia on the cardiorespiratory and the salivary conditional components of an alimentary conditional reflex ([6]) was done in order to solve both questions.

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Methods

All the experiments were performed in a chamber of conditional reflexes, already described by its author (1).

In two male dogs, one weighing 24 kg, of a sanguine temperament, with a strong excitatory tendency, named "Palomo"; the other one weighing 23 kg, of a phlegmatic temperament, named "Pinto", 140 experiments were made in each one, during which: the salivary secretion, tachycardia and tachypnoea produced by the ingestion of food was conditioned to the sound of a metronome; the differentiation of a metronome of double frequency was obtained; the conditional reflex and the differentiation were tried at diverse intervals of the injection of 0.2 mg/kg of cyanide. When the conditional reflex disappeared, the conditioning of salivary secretion to a tactile stimulus, to an electrical vibrator in an improvised chamber, and to a bell (this, one year later), were intended.

The salivary record was made by means of a chronic fistula of the parotid gland's duct.

Cyanide was injected into the peritoneal cavity by a system of remote injection already described (1) similar to that of Teitelbaum & Gantt (14).

To register the cardiac reactions, an electrocardiograph was used, directly recording on a kymograph (2). To register the respiratory reactions an oilskin pneumograph was used conected to a Marey's capsule.

Results

To establish an alimentary conditional reflex, it was necessary to know the unconditional alimentary reaction.

a) Unconditional alimentary reaction.

Ten determinations were made in each dog. In "Palomo", the ingestion of food, produced an average salivary secretion of 87 drops (1 drop = 0.05 cc), with an average latency of 14.3 sec, and an average duration of 90 sec. This secretion was accompanied by a raise in cardiac frequency, averaging 23 beats per minute (b/m), reaching its maximum before starting the secretion and decreasing gradually. The respiratory reaction was not measured in this dog, due to the rough movements it realized while eating.

In "Pinto", the average secretion was of 93 drops, with a latency of 6.1 sec and a duration of 120 sec; the average increase in respiratory frequency was of 7 r/m, and the average increase in cardiac frequency was 17.1 b/m (fig. 1 A).

b) Conditioning of the alimentary reaction to the sound of a metronome of 70 beats per minute (M1).

The M1 was started 10 sec before the apparition of food and was continued until 5 sec after the food appeared; in other words, the conditional reflex was of "short delay" (13, 12). The advantage of using a conditional reflex of short delay (10 sec) is that it provides a constant control of the conditional activity, without the necessity of applying only the conditional stimulus. All the mentioned values concerning the con-

ditioned activity, refer to the reactions occuring during the 10 sec of delay.

At the beginning no reaction was produced by the metronome;

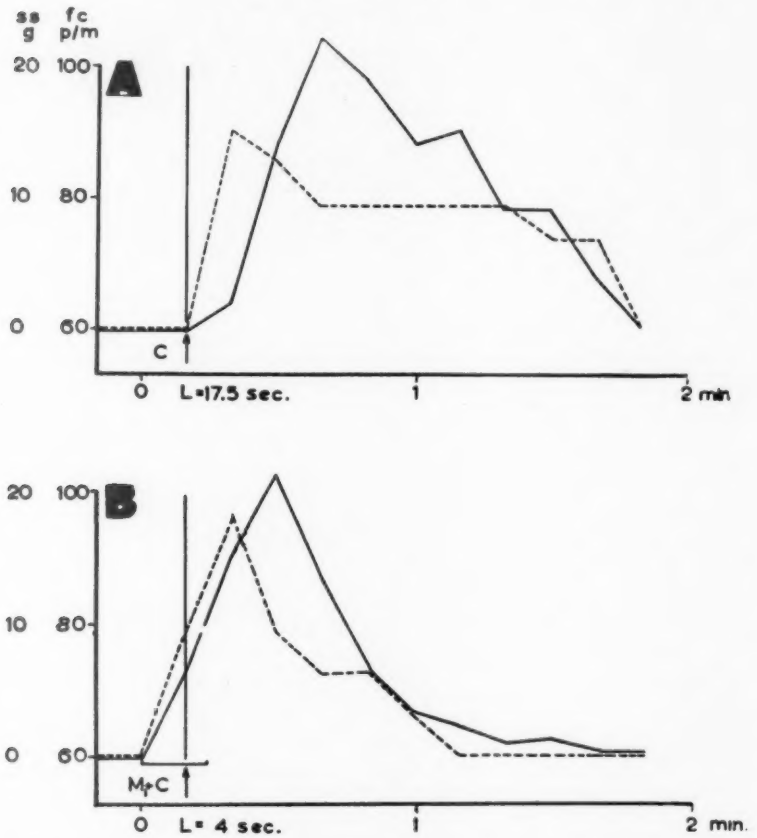

FIG. 1. — *Male dog: "Pinto", chamber of conditional reflexes. Temporal development of the reactions produced by the ingestion of food (A) and by the application of the conditional metronome accompanied with food (B). Abscissae: time in minutes; ordinates: salivary secretion (ss) in drops (g) and cardiac frequency (fc) in beats per minute (p/m). Full line: salivary secretion; broken line: cardiac frequency. C: feeding through; M1 ¡ + C: conditional metronome plus feeding through; L: latency of the salivary secretion starting at 0 in the scale time. The arrow and the vertical line indicate the moment the feeding through appeared.*

after the 9th reinforcement, it started to produce a raise in cardiac frequency and respiratory changes; after the 13th reinforcement the conditional salivary secretion appeared, and 20 reinforcements were needed to stabilize the conditional reflexes.

The values of the conditional reflexes during the first 25 reinforcements were: in "Palomo", 8.3 drops with 3 sec of latency (average conditional salivary secretion) and 9.5 b/m (condition average raise in cardiac frequency); in "Pinto", 6.7 drops with 4 sec of latency and 8 b/m. In the following 200 reinforcements, the average conditional values increased to 9 drops and 12.5 b/m in "Palomo", and 8.6 drops and 13 b/m in "Pinto".

In fig 2 A appears a kymographic record in which the cardiac and salivary conditional reflexes can be observed.

The temporal developments of the cardiac and salivary reactions once the conditional reflex was established are illustrated, in fig 1 B, the conditional effects can be appreciated at the left of the vertical line indicating the apparition of food.

Once the cardiorespiratory and salivary reactions were obtained, a differentiation was established.

c) Differentiation of the sound of a metronome of 140 b/m (M2).

The M2 that had never been accompanied with food, produced in its first applications: tachycardia, respiratory changes and salivary secretion ("irradiation" of the conditional reflex ([11], [12]) (fig 2 B).

In succesive applications of M2 without food, alternating with reinforcements (M1 plus food), the conditional salivary secretion produced by M2 decreased till it completely disappeared, whilst the average conditional secretion obtained with M1 instead of decreasing, increased (from 8.3 to 9 drops in "Palomo"; from 6.7 to 8.6 drops in "Pinto"). The conditional tachycardia produced by M2 did not disappear when stimulus without food was repeated. The average conditional tachycardia for the applications of M2 that did not produce salivary secretion had a value equivalent to 70 % of the tachycardia produced with M1.

The above results indicate that the establishment of a differentiation between M1 and M2 was obtained for the conditional salivary secretion but not for the cardiorespiratory conditional component.

When after a certain time of applying M2, M1 was applied, the latter did not produce the conditional salivary secretion, but it did produce conditional tachycardia and tachypnoea; this means, that the inhibition produced by the differentiated stimulus eliminated only the salivary conditional reflex, without affecting the cardiac and respiratory conditional reflexes.

d) Action of the intraperitoneal injection of 0.2 mg/kg of cyanide, upon the alimentary conditional reflex.

M1 was applied, reinforced with food, before the injection of cyanide, and at different intervals after it, to obtain data in different moments of the temporal development of the histotoxic anoxia.

Before the injection of cyanide, M1 always produced conditional tachycardia, tachypnoea and salivary secretion; when food appeared, the dog approached it inmediately (normal motor conditional reflex) and ate it avidly.

Between 1 to 5 minutes after the injection of cyanide, M1 did not produce conditional salivary reaction, but it did produce conditional tachycardia and tachypnoea; when food appeared, the dog approached it (normal motor conditional reflex) and ate it avidly, producing normal

unconditional salivary secretion. In other words, in this first phase, only the salivary conditional reflexes were inhibited, not affecting the cardiorespiratory and motor conditional reflexes.

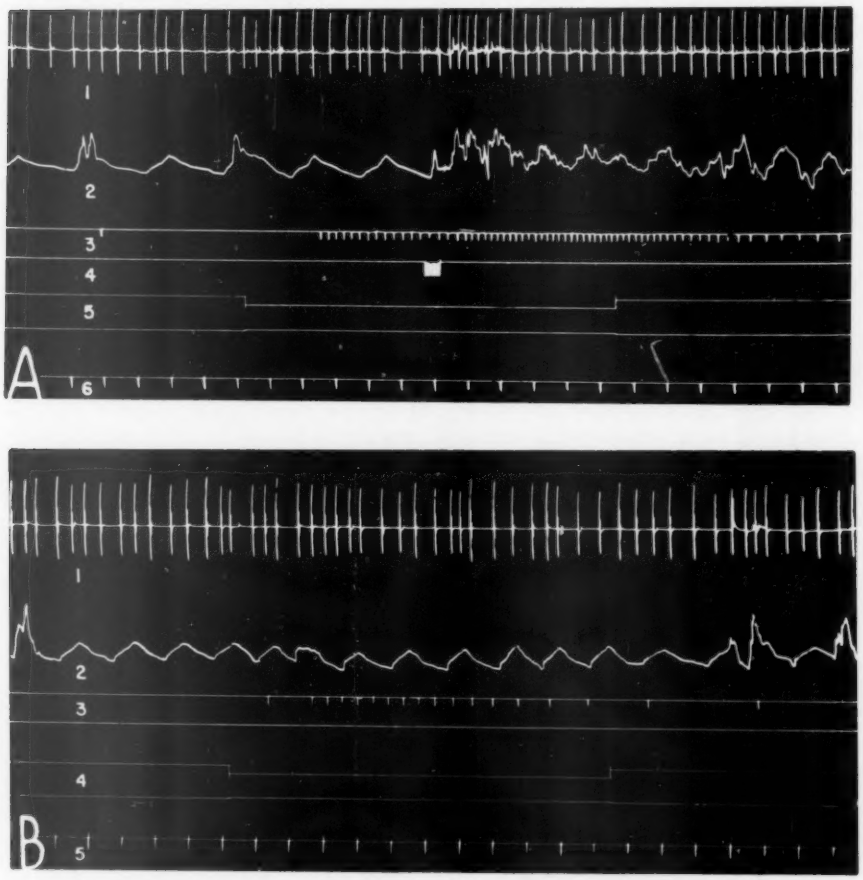

FIG. 2. — *Male dog: "Pinto", chamber of conditional reflexes. Kymographic recording of the effect produced by the conditional metronome (A) and the differentiated metronome in its first applications (B). The records are: in A,* 1. - *Electrocardiogram;* 2. - *Pneumogram;* 3. - *Salivary secretion;* 4. - *Application of the feeding through;* 5. - *Application of the metronome;* 6. - *Time: 2 sec. In B: 1, 2, 3, the same as in A;* 4. - *Application of the metronome;* 5. - *Time: 2 sec.*

Five to ten minutes after the injection, M1 again produced the conditional salivary secretion, but when the food appeared, the dog did not approach it (motor conditional reflex completely inhibited); nevertheless, the conditional salivary secretion was prolonged during 20 sec

after the apparition of food. The cardiac and respiratory conditional reflexes were also unaffected in this phase.

After 10 minutes of the injection, a third phase appeared, in which the motor and the salivary conditional reflexes were completely inhibited; the dog did not approach the food, nor produced any secretion, neither conditional nor direct. Nevertheless, neither the cardiac nor the respiratory conditional reflexes were inhibited in this phase.

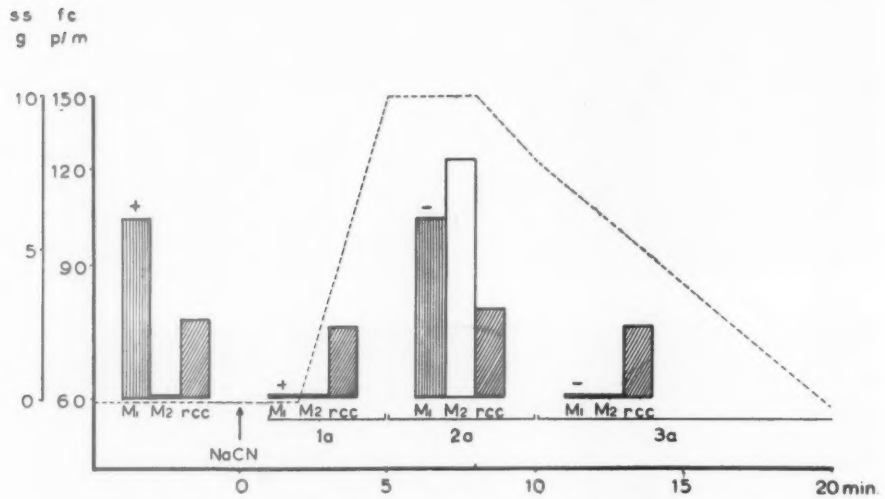

FIG. 3. — *Male dog: "Pinto", chamber of conditional reflexes. Relation between the tachycardia produced by cyanide with the three phases of inhibition that same cyanide produces. Abscissas: time in minutes; ordinates: logarithmic scale of cardiac frequency (fc) in beats per minute (p/m) and linear scale of salivary secretion (ss) in drops (g). M1: conditional metronome; M2: differentiated metronome; rcc: cardiac conditional reflex. The arrow indicates the moment at which the intraperitoneal injection of 0.2 mg/kg of sodium cyanide was applied. (Detailed explanation in the text).*

In fig 3 the temporal relations between tachycardia produced by cyanide and the three phases of inhibition described can be seen: the first phase of inhibition starts one minute before the initiation of tachycardia, and the third phase still persists when tachycardia has already disappeared; in other words, the inhibition produced by cyanide has a lower threshold and a greater inertia than the tachycardia also produced by cyanide. (The threshold for the tachycardia produced by cyanide in an unanesthetized dog is 0.0003 to 0.003 mg/kg by intraperitoneal route; when diluted in at least one litre of blood, it would reach a concentration of 0.0003 to 0.003 μg/kg/cc).

e) Action of cyanide upon the differentiation.

The M2, that before the injection of cyanide produced no salivary secretion, when applied 5 to 10 minutes after the injection (during the

second phase of inhibition) again produced secretion as before the differentiation; meaning that the histotoxic anoxia "disinhibited" the differentiation during the phase in which the salivary conditional reflex reappeared. The M2 produced no salivary secretion in the first nor in the third phase (fig 3).

From the results already exposed it can be concluded that the inhibition caused by histotoxic anoxia eliminates the cortical activity responsible for the salivary and motor conditional reflexes, but it does not affect the cortical activity in relation with the cardiorespiratory conditional reflexes. It must be remembered, that the inhibition produced by the differentiation acts in similar form.

It was interesting to determinate if the inhibition produced by the histotoxic anoxia could be conditional if the cyanide injections were repeated a sufficient number of times.

f) Conditioning of the inhibition produced by cyanide.

After 3 injections of cyanide, the conditional reflex disappeared completely, and it was necessary to reinforce it 9 times (two days) in "Palomo" and 19 times (4 days) in "Pinto" to obtain its reestablishment.

Once the salivary conditional reflex was recovered, the action of cyanide upon it was tried again; after 10 injections the salivary conditional reflex disappeared completely, and its recuperation was not obtained even after 40 reinforcements (20 days) were made, being more than those needed to form it the first time. The average secretion of these 40 reinforcements was 0.5 drops in "Palomo" and 0.9 drops in "Pinto". The cardiorespiratory conditional reflexes were not inhibited.

The fact that the inhibition of the salivary conditional reflex "persisted" 20 days after the last injection of cyanide, suggested a conditioning of such inhibition to the "stereotype" of the chamber of conditional reflexes; that is, that the chamber was converted into a stimulus capable of provoking the inhibition of the salivary conditional reflex; this conditional inhibition did not affect the cardiorespiratory conditional reflexes.

If the inhibition produced by cyanide had been conditional to the chamber, it could be expected that the tachycardia produced by cyanide would have also been conditional, that is, that the entrance to the chamber should by itself produce an elevation in the cardiac frequency of the dogs. Actually, the average of the basal cardiac frequency of the dogs after 10 injections of cyanide is 50 % higher than before the application of cyanide.

To determine if the conditional inhibition produced by the chamber affected only the auditory stimulus, the conditional of the alimentary reaction to a different stimulus (tactile) was tried. In 30 reinforcements the salivary conditional reflex could not be formed, the average secretion being 0.5 drops in "Palomo" and 0.4 drops in "Pinto" Nevertheless, the cardiac and respiratory components were conditional to the tactile stimulus.

To determine if the partial modification of the stereotype caused the conditional inhibition to diminish or disappear, a chamber of conditional reflexes was improvised in a different room than the one in

which all the preceding experiments were made; in this chamber the conditioning of the alimentary reaction to an electric vibrator was tried. In 40 reinforcements the salivary conditioning was not obtained, obtaining only an average secretion of 0.3 drops in "Palomo" and 0.7 drops in "Pinto". The conditioning of the cardiorespiratory component was obtained.

g) Persistency of the conditioning of the inhibition produced by cyanide.

After 15 months of the last injection of cyanide, the conditioning of the salivary secretion to a new stimulus (bell) was again tried; the results of this series of experiments were different in the two dogs.

In "Palomo", after the third reinforcement, the bell started to produce a conditional salivary secretion, and after the eight, the salivary conditional reflex was stabilized. The average conditional secretion after the eight reinforcement was of 8 drops with a latency of 2 sec, the cardiac component of the alimentary reaction was also conditional, obtaining an average conditional raise of 8.7 b/m.

In "Pinto", the salivary conditional reflex could not be formed after 40 reinforcements, a conditional secretion of only 0.5 drops in average being obtained.

It may be deduced from these results that the inhibition produced by cyanide can be conditional to the stereotype of the chamber, giving as a result the conditional inhibition that prevents the realization of the salivary conditional reflex already acquired, and the formation of new salivary conditional reflexes; but it does not prevent the obtention of the cardiorespiratory conditional reflexes already formed, nor the obtention of new ones. The conditional inhibition persisted in both dogs after three months of the last injection of cyanide, but only in the phlegmatic dog it persisted after 15 months.

Discussion

The study of the action of histotoxic anoxia upon the alimentary conditional reflexes, has confirmed the inhibition of the salivary conditional reflex observed by other authors using anoxemic anoxia (Malmejac & Plane, [7], [8], [9], [10]; Gantt, [5]) but it has also demonstrated that this inhibition presents in its temporal development, three perfectly defined phases. These three phases (1st: inhibition of the salivary conditional reflex; 2nd: inhibition of the motor conditional reflex and reappearance of the salivary conditional reflex, 3rd: inhibition of both conditional reflex; 2nd: inhibition of the motor conditional reflex and reappearance progressive establisment of natural sleep; in other words, the inhibition produced by hystotoxic anoxia has a similar temporal development to the inhibition produced during sleep.

The cardiac and respiratory conditional reflexes did not disappear in any of the three phases of inhibition produced by histotoxic anoxia. This means that the inhibition produced by cyanide at the doses employed, does not affect the reflexes whose effectors belong to the cardiorespiratory functional system. This explains why it is possible to obtain the conditioning of the cardiorespiratory responses provoked by

cyanide ([8], [13]) in spite of the intense inhibition produced by it upon the cortical functions related to the motor and salivary reflexes.

The fact that the inhibition produced by the application of differentiated stimulus, also affects only the salivary conditional reflex, and not the cardiac and respiratory conditional reflexes is interesting, because it indicates that the inhibition obtained with cyanide presents similar characteristics to the cortical physiological inhibition (which Pavlov called: "internal inhibition").

The increase in the salivary conditional reflex obtained by Malmejac & Plane with a slight anoxemic anoxia, was never observed with the hystotoxic anoxia. The "adaptation phenomena" obtained by these authors, consisting in the slow diminution of the inhibition produced by anoxemic anoxia with the repetition of the experiment, were also not observed; instead, the repetition of the histotoxic anoxia led to the conditioning of the inhibition it produces This difference is probably due to the fact that the anoxemic anoxia may be compensated by the cardiorespiratory reactions it produces, which when conditioned increase their efficiency, the compensation of the oxygen insufficiency being each time more premature and complete, causing the inhibition to appear each time with less intensity, until its disappearance after a certain number of repetitions. On the contrary, the histotoxic anoxia cannot be compensated by the cardiorespiratory reactions, thus explaining the differences between these studies and those presented by Malmejac and Plane.

The conditioning of the inhibition produced by histotoxic anoxia indicates that this inhibition was not caused by the direct action of cyanide upon the cortical cells, but by the action of the said cyanide upon some afferent chemoreceptor. Gantt ([4]), demonstrated that the reactions produced by the direct stimulation of the cerebral cortex cannot be conditional. In other words, it seems that the inhibition produced by the histotoxic anoxia is the physiological response of the cerebral cortex to the signals proceeding from the chemoreceptors (the arrival of such signals to the cerebral cortex has been already demonstrated, [8], [13]).

The fact that the inhibition produced by cyanide presents similar characteristics of the physiological inhibition (sleep, differentiation) and the fact that it starts when the concentration of cyanide in the blood is less than 0.003 μg/kg/cc (threshold of the tachycardia) supports the hypothesis that it is a physiological response of the cerebal cortex and not a toxic phenomenon produced by the action of cyanide upon the cortical neurons.

If the interpretations given to these facts are true, the functional scheme of the cortical response to anoxia must be the following:

The oxygen insufficiency stimulates the chemoreceptors producing signals that are conducted to the nervous centers. The response of the cerebral cortex to these signals is an inhibition that eliminates the motor and salivary conditional reflexes (and perhaps others that were not tried), thus diminishing the oxygen requirements of the organism, but not affecting the cardiorespiratory conditional reflexes which tend to diminish the oxygen deficiency. The repetition of the histotoxic anoxia

brings forth as a consequence the conditioning of the cardiorespiratory reactions and the cortical inhibition.

Conclusions

1. — The arrival to the cerebral cortex of the chemoreceptors signals produces the inhibition of the salivary and motor conditional reflexes, without affecting the cardiac and respiratory conditional reflexes.

2. — The cortical inhibition produced by the histotoxic anoxia may be conditional; the resulting conditional inhibition causes the already formed salivary reflex to disappear, and prevents the obtention of new salivary conditional reflexes, but does not affect the cardiorespiratory conditional reflexes already existing, nor prevents the formation of new ones.

Summary

In two dogs of a different temperament (one excited, the other one inhibited) isolated in a chamber of conditional reflexes provided with a system of remote injection and with electrocardiographic and pneumographic recordings, 260 experiments were realized.

The action of histotoxic anoxia upon the cortical activity was studied, using as an indicator an alimentary conditional reflex and its differentiation.

The salivary secretion produced by food was accompanied by tachycardia and tachypnoea; when the alimentary conditional reflex was formed the cardiac and respiratory components were also conditional; when the differentiation of the salivary conditional reflex was established, the cardiac and respiratory conditional reflexes did not differentiate.

The intraperitoneal injection of 0.2-mg/kg of cyanide produced a cortical inhibition which presented three phases in its temporal development:

1st. — From 1 to 5 minutes after the injection, inhibition of the salivary conditional reflex. This phase started approximately 1 minute before the tachycardia produced by cyanide.

2nd. — From 5 to 10 minutes after the injection, inhibition of the motor conditional reflex, and reappearance of the salivary conditional reflex; disinhibition of the differentiation.

3rd. — After 10 minutes of the injection, inhibition of the motor and salivary conditional reflexes.

In none of the three phases were the cardiac and respiratory conditional reflexes affected.

The inhibition produced by the histotoxic anoxia was considered as a conditioning to the "stereotype" of the chamber in which cyanide was injected; the resulting conditional inhibition eliminated the salivary conditional reflex, but not the motor, cardiac and respiratory conditional reflex, the conditional inhibition produced by the chamber, prevented the formation of new salivary conditional reflexes, but it did not impede the obtention of motor and cardiorespiratory conditional reflexes.

The conditioning of the inhibition produced by cyanide persisted in both dogs after three months of the last injection, and in one of them (of an inhibited temperament) after 15 months.

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REFERENCES

(1) ALVAREZ-BUYLLA, R.: Descripción de una cámara para el estudio de reflejos condicionados. *Ciencia Mex.*, 1949, *10*, 291.
(2) ALVAREZ-BUYLLA, R., BECKWITH, J.: Equipo electrónico para el estudio de potenciales bioléctricos. *Ciencia Mex.*, 1951, *11*, 163.
(3) ALVAREZ-BUYLLA, R., RUSSEK, M.: Intervención de la corteza cerebral en la integración de una reacción vegetativa. Congreso del IV Centenario de la U.N.A.M. 1951. *Act. Physiol. Latinoamer.*, 1952, *2*, 119.
(4) GANTT, H. W.: Essential anatomical structures of the reflex arc for establishing of conditioned reflexes. *Amer. J. Physiol.*, 1934, *119*, 313.
(5) GANTT, H. W.: Anoxia on conditional reflexes in dogs. *Fed. Proc.*, 1949, *8*, 53.
(6) GANTT, H. W., HOFFMAN, H.: Conditioned cardiorespiratory changes accompanying conditioned food reflexes. *Amer. J. Physiol.*, 1940, *129*, 360-61.
(7) MALMEJAC, J., PLANE, P.: Astivité corticale et déficit en oxigène. Etude a l'aide du refléxe conditionnél. *Algeric med.*, 1951, *55*, 969; *C. R. Soc. Biol.*, *145*, 1136.
(8) MALMEJAC, J., PLANE, P.: Effects de l'anoxie sur les aptitudes fonctionéles corticales. Reaction d'adaptation. *C. R. Soc. Biol.*, 1951, *145*, 1163.
(9) MALMEJAC, P, PLANE, P.: Etude a l'aide du reflex conditionnél salivaire chez le chien, de dysfonctionnement nerveux superieurs a des agressions diverses. Aspects techniques et physiologiques. *Bull. Acad. Nat. med.*, 1952, *1*, 24.
(10) MALMEJAC, J., PLAN, P.: Influence of oxygen want on functional cortical fitness. *J. Aviat. Med.*, 1952, *23*, 177.
(11) PAVLOV, I. P.: Lectures on Conditioned reflexes. *Inter. Pub.*, 1928.
(12) PAVLOV, I. P.: Los reflejos condicionados. *Ed. Pavlov*, México.
(13) RUSSEK, M.: Conocimiento de la taquicardia producida por la anoxia histotóxica. En prensa.
(14) TEITELBAUM, H., GANTT, H. W.: A method of intravenous injection from a distance in conditioned reflexes studies. *Science*, 1952, *113*, 603.

A DESCRIPTION OF THE CIRCULATORY DYNAMICS IN THE HEART AND LUNGS OF PEOPLE AT SEA LEVEL AND AT HIGH ALTITUDE BY MEANS OF THE DYE DILUTION TECHNIQUE

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INTRODUCTION

THE DYE DILUTION method for measuring cardiac output ([6], [13]) has proved valuable in describing the central circulation. This technique has been widely used for the determination of cardiac output, mean circulation time and intrathoracic blood volume as described by Hamilton.

In 1951, Newman et al ([10]), made an analysis of the factors shaping the time concentration curve and described a formula which could be used for the determination of "Central Volume". This volume would correspond to the amount of blood in the lungs. In a later publication, Newman et al ([7]) described animal experiments designed to prove the validity of their theoretical assumptions.

The method in a single experiment permits the measurement of a) circulation time; b) cardiac output; c) intrathoracic blood volume (Hamilton); d) central volume (Newman) and e) total blood volume.

The purpose of our study is to describe the hemodynamics of the circulation in the heart and lungs of people at sea level and at high altitude by means of the dye dilution technique. It has been demonstrated that Peruvian natives born and living at a high altitude have a larger chest ([3], [8]) and a greater vital capacity than men living at sea level, consequently, we thought that it might be possible that the natives at high altitudes might have a greater quantity of blood in the lungs and that the dye dilution technique would be a useful tool in its determination.

We are not aware of any systematic work in normal males and females using Newman's calculation, so we have also included in this work

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a group of normal females living at sea level to be compared with a similar male group.

MATERIAL AND METHODS

We have selected for this study a group of 45 normal people. Twenty sea level men, 15 sea level women and 10 male natives of the Peruvian Andes who belong to a race who have lived at high altitudes since prehistoric times.

The criteria for selection were: history, physical examination, electrocardiogram, chest X rays, and normal hematological findings at sea level. We were careful to avoid cases of pneumoconiosis, a common finding in the natives of the Andes who work in the mining camps. The sea level group included people who have never lived at high altitude. The age of the male group varied from 21 to 26, and the female group from 16 to 27. The high altitude group included 10 Peruvian male natives who had never been at sea level and who were residents of Morococha or nearby altitudes; the age of this group varied from 18 to 30.

The places of study were: Lima (sea level) located at 150 meters above sea level and Morococha (high altitude) located at 4 540 meters (14 900 feet) above sea level.

The experimental procedure was the same described by Hamilton [9] with some modifications. The apparatus for the serial collection of arterial blood was the same as described by Newman [10]. All subjects arrived in the morning at the laboratory in overnight fasting conditions with the exception of the high altitude natives who had taken a very slight breakfast, which consisted of a piece of bread taken about four hours before the experiment. The experiment was made with the person lying on a bed after a minimum of 30 minutes rest. In all cases, after administration of procaine anesthesia, the femoral artery was punctured with a number 18 needle attached to a short piece of plastic tube. The same tube was used for all the experiments, and it served the purpose of collecting the blood in small test tubes. Two cm^3 of a one percent solution of Evans blue were measured in a 3 cm^3 syringe lubricated with mineral oil; the syringe containing the dye attached to a number 20 needle was weighed in a precision balance before and after the injection was given.

The experimental procedure consisted of the injection of Evans blue into an antecubital vein and the simultaneous collection of arterial blood at two second intervals. Before the injection, blood for blanks and hematocrits was collected from the arterial needle. Ten minutes after the injection of T-1824 a venous blood sample was drawn for plasma volume determinations.

The analysis were made in a Coleman Jr. spectrophotometer at 620 $m\mu$ in serum, using a microtechnique by means of melting point capillary tubes. Hemolysis was avoided in almost all cases. In the few samples where hemolysis was present a correction was made for the presence of hemoglobin by means of a new reading at 540 $m\mu$. The correction factor proved to be negligible. Hematocrits were centrifuged

TABLE I

SEA LEVEL MEN

NAME	AGE	W	SA	AT	MCT	BUT	Ht.	CI	CV	ITV	TBV	$\frac{CV}{TBV} \times 100$	$\frac{ITV}{TBV} \times 100$
		Kg	m^2	sec/m^2	sec/m^2	sec/m^2	%	$L/m/m^2$	L/m^2	L/m^2	L/m^2	%	%
E.C.	26	65.0	1.78	10.4	17.5	6.0	45.0	3.9	0.47	1.99	2.79	16.8	71.6
A.G.	26	68.5	1.76	9.7	17.7	5.5	45.5	3.4	0.38	1.74	2.83	13.5	61.6
L.M.	21	66.5	1.75	8.6	14.8	4.4	46.3	3.9	0.42	1.67	2.56	16.5	65.2
J.S.	22	67.5	1.83	8.2	14.8	5.3	46.5	3.3	0.36	1.47	2.47	15.0	60.3
J.H.	25	70.0	1.82	8.2	16.1	5.1	46.0	3.6	0.55	1.73	2.71	20.3	63.9
G.M.	24	77.0	1.92	5.4	11.8	4.9	47.1	4.1	0.31	1.55	2.61	12.0	59.5
L.N.	22	75.8	1.90	9.5	16.2	4.5	44.1	3.1	0.38	1.60	2.92	13.2	54.8
J.S.	24	52.8	1.56	10.3	19.8	6.5	46.0	3.2	0.33	1.67	2.69	12.2	69.0
E.G.	27	52.8	1.56	13.2	21.2	4.9	46.5	3.8	0.43	2.08	2.83	15.2	73.7
A.C.	22	59.5	1.63	8.8	15.1	5.0	46.8	4.0	0.34	1.67	2.36	14.6	70.8
G.C.	22	60.5	1.67	8.7	15.9	5.3	46.2	3.7	0.40	1.62	2.90	13.9	55.8
A.C.	23	55.6	1.60	10.2	16.2	5.7	46.0	3.2	0.21	1.40	2.60	7.9	57.9
A.L	23	55.8	1.52	10.2	17.8	5.1	50.0	3.3	0.42	1.49	2.53	16.3	57.7
J.J.	22	55.6	1.63	9.1	14.4	4.5	46.0	4.4	0.28	1.71	2.43	11.7	70.4
G.L.	22	64.2	1.74	11.7	19.8	4.7	42.5	3.3	0.56	1.92	2.31	24.4	83.1

NAME	AGE	W	SA	AT	MCT	BUT	Ht.	CI	CV	ITV	TBV	$\frac{CV}{TBV} \times 100$	$\frac{ITV}{TBV} \times 100$
		Kg	m^2	sec/m^2	sec/m^2	sec/m^2	%	$L/m/m^2$	L/m^2	L/m^2	L/m^2	%	%
J.S.	25	74.1	1.85	7.0	11.2	2.9	46.5	4.1	0.31	1.43	2.76	11.1	52.1
G.W.	22	62.5	1.72	11.3	21.5	6.7	48.0	2.4	0.49	1.50	2.62	28.6	57.2
L.R.	23	70.0	1.85	7.9	15.0	5.3	47.1	3.3	0.35	1.51	2.67	13.0	56.7
C.R.	21	57.4	1.63	11.9	20.5	6.8	44.8	3.5	0.46	1.93	2.28	13.9	58.8
J.F.	23	55.5	1.60	6.2	12.1	5.0	42.5	3.8	0.35	1.27	2.40	14.5	56.0
MEAN			1.72	9.3	16.5	5.2	46.0	3.56	0.39	1.65	2.66	15.2	62.3
STANDARD DEVIATION				1.94	3.02	0.89	1.69	0.46	0.09	0.21	0.23	4.70	8.40

W.: Weight

S.A.: Surface Area

A.T.: Appearance time

B.U.T.: Build up time

Ht.: Hematocrit

C.I.: Cardiac Index

C.V.: Central Volume

L.T.V.: Intra Thoracic Volume

T.B.V.: Total blood volume

at 3 000 rpm during 30 minutes at sea level and during 60 minutes at high altitude (high hematocrits).

CALCULATIONS

The amount of dye given was calculated by weight differences of the syringes. The dilution curve was plotted on semilogarithmic paper.

Circulation times. — The following times were measured: a) appearance time: from the time of injection to the first appearance of dye; b) build-up-time: from the begining of the dilution curve to the apex of the curve; c) mean circulation time: by Hamilton's formula ([2])

$$M = \frac{T_1C_1 + T_2C_2 + \ldots + TnCn}{C_1 + C_2 + \ldots + Cn}$$

M is the T — coordinate of the center of gravity of the curve; C_1, C_2, ... Cn, are the concentration readings at times T_1, T_2, ... Tn. The mean circulation time is the time from the middle of the injection period to M.

Cardiac output. Hamilton ([6]) $F = \frac{I \times 60}{CT}$

I is the amount of dye given: CT is the area of the curve;
F is expressed in liters per minute.

Intrathoracic blood volume. Hamilton ([2]) $= \frac{F \times MCT}{60}$

F is cardiac output in liters per minute and MCT mean circulation time.

Central volume. Newman's formula ([10]) $S = \frac{V}{F}$

S is slope; F is flow per unit time; V is central volume.

Total blood volume. $= \frac{I \times 100}{C_{10} \times (100\text{-Ht})}$

I is the amount of dye given; C_{10} dye concentration at the end of ten minutes; (100-Ht) = plasmatocrit. A small correction was made to compensate for the amount of dye lost during the arterial collection. Hematocrits were not corrected for trapped plasma.

RESULTS

All experimental data is summarized in tables I, II and III. Times and volumes have been corrected to surface area; this correction is important in comparing the sea level and high altitude groups since natives of the altitude are shorter and lighter than people of sea level.

As we are interested in the comparison of men and women at sea level and men of the altitude with men of sea level, we have arranged our data on table IV in such a way as to show the differences between the mean values of the groups. Table IV also shows the values of P. We have taken into consideration only P values less than 0.05.

The reader who is not familiar with the work at high altitude might be surprised to find the wide variation of data in the high altitude group, but this is a common finding in an enviroment in which the arterial saturation rests on the steep side of the oxygen dissociation curve.

TABLE II

SEA LEVEL WOMEN

NAME	AGE	W	SA	AT	MCT	BUT	Ht.	CI	CV	ITV	TBV	$\frac{CV}{TBV} \times 100$	$\frac{ITV}{TBV} \times 100$
		Kg	m^2	sec/m^2	sec/m^2	sec/m^2	%	L/m/m^2	L/m^2	L/m^2	L/m^2	%	%
M.V.	22	49.8	1.52	5.3	11.1	3.9	41.5	4.9	0.36	1.37	2.26	15.9	60.5
Y.S.	26	50.7	1.46	15.5	23.0	5.6	40.0	2.6	0.37	1.45	2.26	16.4	64.7
B.R.	26	67.4	1.67	9.6	16.0	4.8	40.0	4.6	0.49	2.03	2.52	19.4	81.1
G.R.	26	50.6	1.53	7.8	14.1	5.2	40.0	3.8	0.39	1.37	2.36	16.4	57.9
L.P.	23	55.3	1.54	6.6	13.9	5.3	40.0	2.8	0.32	0.99	2.70	14.7	45.7
A.V.	22	45.6	1.39	7.3	14.0	4.4	40.6	3.8	0.33	1.22	1.99	18.4	61.2
L.M.	24	47.5	1.41	8,1	14.0	4.8	41.5	4.4	0.35	1.45	2.00	17.4	72.2
D.M.	25	52.0	1.51	5.8	11.7	5.8	40.0	3.1	0.22	0.91	2.46	10.7	44.5
V.C.	18	49.8	1.44	6.9	14.9	6.9	37.0	2.9	0.30	1.02	2.02	14.8	50.4
L.B.	22	54.0	1.54	7.1	14.5	5.9	40.5	3.3	0.44	1.24	2.33	19.0	53.1
J.E.	23	65.0	1.63	8.7	15.3	5.0	36.0	3.2	0.32	1.35	2.55	12.6	35.2
T.E.	16	50.3	1.43	10.1	18.0	4.3	38.0	3.4	0.38	1.46	2.58	14.6	56.6
T.E.F.	16	53.3	1.49	11.1	19.4	5.0	41.0	3.8	0.48	1.85	2.50	19.0	74.0
J.S.	17	48.5	1.39	10.2	16.9	4.4	41.0	4.8	0.46	1.90	2.40	19.0	79.1
D.Y.	25	50.2	1.54	4.7	12.2	4.7	40.5	4.3	0.50	1.34	2.75	19.2	48.6
MEAN			1.50	8.3	15.3	5.1	39.8	3.71	0.38	1.40	2.34	16.5	60.0
STANDARD DEVIATION				2.73	3.10	0.80	1.60	0.75	0.08	0.30	0.23	2.64	13.40

TABLE III

HIGH ALTITUDE MEN

NAME	AGE	W	SA	AT	MCT	BUT	Ht.	CI	CV	ITV	TBV	$\frac{CV}{TBV} \times 100$	$\frac{ITV}{TBV} \times 100$
		Kg	m^2	sec/m^2	sec/m^2	sec/m^2	%	$L/m/m^2$	L/m^2	L/m^2	L/m^2	%	%
D.T.	18	53.8	1.52	9.9	16.7	5.6	60.9	4.2	0.35	1.79	3.32	10.6	54.0
C.S.	22	56.4	1.57	10.4	22.9	10.4	69.3	3.0	0.54	1.83	3.27	16.5	55.8
L.R.	23	52.3	1.47	11.2	23.3	6.4	60.6	3.9	0.77	2.21	3.71	24.5	59.5
F.U.	25	59.7	1.65	10.9	15.1	7.5	55.7	4.0	0.65	1.68	3.85	16.9	43.6
G.Ch.	21	53.0	1.50	9.8	16.2	4.5	54.9	4.8	0.54	1.95	3.18	17.0	61.2
L.I.	28	53.7	1.51	15.0	27.5	7.8	53.0	4.7	1.06	3.23	3.58	29.6	90.2
P.V.	22	56.2	1.54	14.3	27.6	9.1	64.7	3.4	0.79	2.39	3.77	21.4	63.3
H.E.	30	54.2	1.52	12.0	24.3	6.6	66.4	3.6	0.85	2.19	3.89	21.9	56.4
B.Z.	23	56.5	1.67	7.3	16.1	6.0	61.3	5.0	0.83	2.23	5.50	23.8	63.7
T.S.	23	58.9	1.60	9.4	16.9	6.1	50.7	3.6	0.33	1.62	2.21	12.3	73.4
MEAN			1.55	11.0	20.7	7.0	59.7	4.02	0.67	2.11	3.63	19.4	62.1
STANDARD DEVIATION				2.29	4.95	1.74	6.07	0.65	0.23	0.47	0.67	5.84	12.50

Discussion

The main purpose of our work has been to study the amount of blood in the lungs by means of Newman's formula. Most other investigators have used Hamilton's procedure (mean circulation time multiplied by cardiac output). If we accept Newman's theory of instanteneous dilution of the dye in the central circulation and its washout governed by the lungs, the mean circulation time has no physiological meaning. We have used both systems of calculations, and figure 1 shows their correlation which is statistically significant ($r = 0.725 \pm 0.04$ S.E.). This

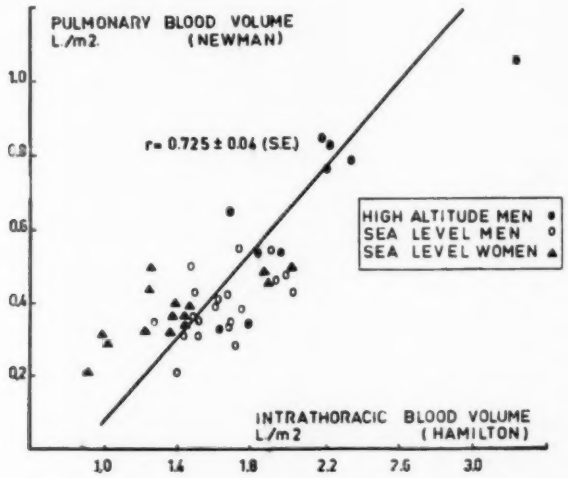

FIG. 1. — *The figure shows the correlation between pulmonary blood volume in liters per square meter (calculated by Newman's formula, from the descending slope of the curve) and intrathoracic blood volume in liters per square meter (calculated from mean circulation time and blood flow according to Hamilton). It can be seen that correlation is good: $r = 0.725 \pm 0.04$ S.E. The high altitude men figures lie in the zone of greater volumes.*

means that either procedure can be used as a relative estimation of thoracic blood volume but, of course, the validity of the absolute figures depends on the validity of either theory. Figure 1 is also very illustrative in showing the great difference in pulmonary blood volume and intrathoracic blood volume between high altitude men and sea level men. It also shows that the correlation between these two volumes seems to be present even in the extremely high values.

Although there are many reports in the literature in which the dye dilution technique has been used, it is difficult to make a comparative study because of the differences in the technique. The majority of authors have used mean circulation time to calculate intrathoracic blood volume and values differ greatly depending on the site of the injection of the dye and the collection of the samples. These differences also

TABLE IV

P VALUES *

	AT	MCT	BUT	Ht.	CI	CV	ITV	TBV	$\frac{CV}{TBV} \times 100$	$\frac{ITV}{TBV} \times 100$
	sec/m²	sec/m²	sec/m²	%	L/m/m²	L/m²	L/m²	L/m²	%	%
Sea level men (means)	9.3	16.5	5.2	46.0	3.56	0.39	1.65	2.66	15.2	62.3
Sea level women (means)	8.3	15.3	5.1	39.8	3.71	0.38	1.40	2.34	16.5	60.0
P				< 0.01			< 0.01	< 0.01		
Sea level men (means)	9.3	16.5	5.2	46.0	3.56	0.39	1.65	2.66	15.2	62.3
High altitude men (means)	11.0	20.7	7.0	59.7	4.02	0.67	2.11	3.63	19.4	62.1
P	0.04	< 0.01	< 0.01	< 0.01	0.04	< 0.01	< 0.01	< 0.01	0.02	

* Only P values < 0.05 are written on the table.

P values calculated by "t test" of Fisher.

indicate that this method of calculation is not an accurate one because of its dependence on mean circulation time.

Sea level group. — Newman's formula has not been widely used as a means of calculating pulmonary blood volume. Our results agree well with those of Schreiner et al ([12]) who have found an average of 13.3 percent of total blood volume as central volume (Newman's formula) in 16 normal subjects. Our sea level figures are 15.2 percent for the male group and 16.5 percent for the female group. There are other figures reported in the same paper which are in accordance with ours: cardiac index 3.83 L/m/sq m; appearance time: 13.1 s; build-up-time:

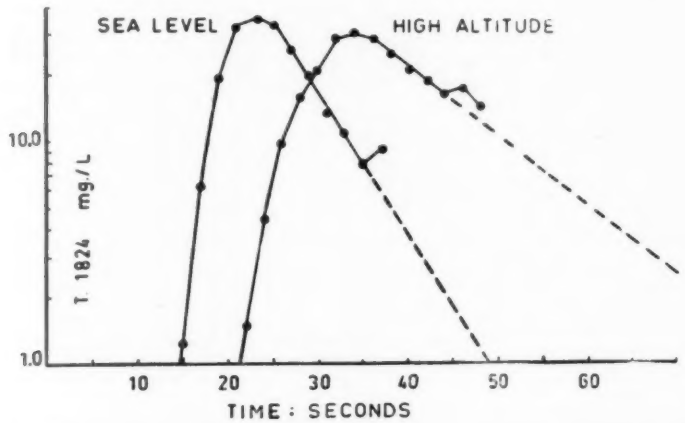

FIG. 2. — *The figure shows a representative curve from a sea level experiment and from a high altitude experiment. The injection uas given at 0 time and it can be seen that the high altitude curve has a longer appearance time and a longer build-up-time. The differences in the slopes are also evident. The amount of dye used in all the experiments was the same.*

7.1 s; do not differ greatly with ours of 3.56, 15.9 and 9.0 respectively (male group).

Chapman et al ([1]) have used the dye dilution technique for the measurement of cardiac output and mean circulation time in a group of 23 young males and 10 young females. As they have used a polyethylene catheter introduced about 30 cm into the vein, their circulation times can not be compared with ours in which a needle was used. The male group cardiac index which gives a mean value of 3.63 ± 0.59 (S.D.) L/m/sq m agrees well with our mean value of 3.56 ± 4.46 (S.D.) in 20 sea level males. Their female group has a mean of 3.50 ± 0.78 (S.D.) slightly lower than ours of 3.71 ± 0.71 (S.D.) in the 15 sea level females. It is interesting to observe that the standard deviation of the mean for both groups of investigator shows the same differences in the male and female group.

Our blood volume results at sea level give a mean of 2.66 ± 0.23

(S.D.) L/sq m for the male group and a mean of 2.34 ± 0.23 L/sq m for the female group, a result somewhat lower than Jasahui's ([3]) made in our laboratory (Lima) in a similar group of normal sea level people and using the standard T-1824 technique for blood volume determinations. Jasahui's results in fifty males are 2.97 ± 0.25 (S.D.) L/sq m and 2.51 ± 0.17 (S.D.) L/sq m in fifty females.

High altitude group. — Our blood volume studies agree very well with previous work done at the Instituto de Biología Andina by Hurtado et al ([4]) who worked at the same altitude (Morococha 14 900 feet) and found a hematocrit of 59.9 ± 5.6 (S.D.) in 32 subjects. Our results are 59.7 ± 6.07 (S.D.). Hurtado's blood volume results give a mean

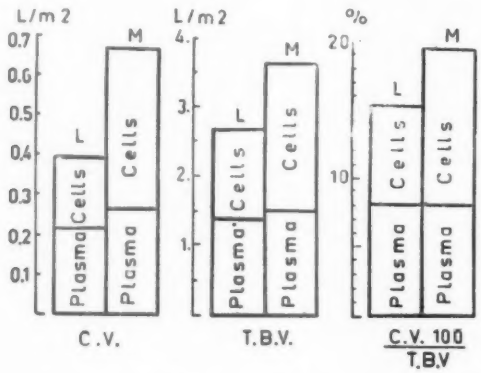

FIG. 3. — *L = Lima (Sea level); M = Morococha (14 900 feet altitude); CV = Central volume (Pulmonary blood volume); TBV = Total blood volume.*
The figure shows the increased pulmonary blood volume and total blood volume of the natives of Morococha. It also shows that the increase in pulmonary blood volume is greater than the corresponding increase in total blood volume, giving a higher percentage of total blood volume as pulmonary blood volume in the altitude.

of 100.3 ± 10.8 (S.D.) cm^3/kg of body weight for total blood volume in 11 persons, using the Evans blue dye. Our results are 96.2 ± 14.2 (S.D.) cm^3/kg of body weight.

The pulmonary blood volume calculated by Newman's formula shows an absolute figure of 0.67 L/sq m which is higher than the male sea level group of 0.39 L/sq m, the difference being statistically significant with a P value less than 0.01. As we were interested in knowing if this difference was only the result of the increased blood volume at the high altitude, we have calculated the pulmonary blood volume as a percentage of total blood volume. The mean value of sea level men is 15.2 percent as compared with 19.4 percent of the high altitude group. The P value equals 0.02 which shows borderline significance. These results are in favor of our hypothesis of a greater blood volume in the lungs of the Peruvian high altitude natives.

The mean circulation times and build-up-times are longer in the high altitude group, and the difference is statistically significant. The longer build-up-time makes the high altitude curves look different from the sea level curves (Figure 2). Newman ([10]) has demonstrated in his experimental work with bottles representing the chambers of the heart that the build-up-time is governed by the size of these bottles. Our results, therefore, would indicate a greater capacity of the heart chambers in our high altitude group if the Newman's hypothesis is correct. Nevertheless, further work must be done to substantiate these initial observation.

Our cardiac index of 4.02 ± 0.65 (S.D.) L/m/sq m is higher than our sea level figure of 3.56 ± 0.46 (S.D.) L/m/sq m. The P value of the difference between the means is 0.04 having a doubtful significance. Previous work at the same altitude has been done by Rotta using the acetylene method in 16 healtly adults born and living in Morococha. His results "showed normal values for the cardiac output, and a very moderate increase in the cardiac index". (No figures are given in the paper). The same author using the Fick procedure (same paper) found in three natives from Morococha a cardiac index of 3.20, 2.60 and 2.64 L/m/sq m definitely lower than his sea level figures done in two controls who showed a cardiac of 4.08 and 4.07 L/m/sq m ([13]). It is evident that further work is needed to clarify these results.

Figure 3 ilustrates the differences between total blood volume and pulmonary blood volume between the men of sea level and the men of the high altitudes. They show clearly that the increased volumes in the altitude depend on a higher red cell mass, a well known fact in anoxic polycythemia.

Summary

The dye dilution technique has been used for the description of the circulatory dynamics in the heart and lungs of people at sea level and at a high altitude. The main purpose of this work has been the measurement of the amount of blood in the lungs in these groups by means of Newman's formula, in order to find out if natives of the altitude have a greater quantity of blood in the lungs. The following calculations have been made: circulation time, cardiac output, intrathoracic blood volume (Hamilton), central volume (Newman) and total blood volume. At sea level the study was carried out in 20 males and 15 females. At high altitude (14 900 feet) the study was carried out in 10 Peruvian male native born and living in the altitude. When values are corrected per square meter of body surface area, they do not differ greatly between males and females at sea level, aside from the well known hematological differences. The high altitude male native group shows longer circulation time, slightly greater cardiac output, greater blood volume, and greater quantity of blood in the lungs. When the amount of blood in the lungs, as calculated by Newman's formula is expressed as a percentage of total blood volume, the high altitude group also shows a higher figure over the sea level group.

REFERENCES

(1) CHAPMAN, C. B., FRASER, R. S.: Studies on the effect of exercise on cardiovascular function. I. Cardiac output and mean circulation time. *Circulation*, 1954, *9*, 57.

(2) HAMILTON, W. F., MOORE, J. W., KINSMAN, J. M., SPURGLING, R. G.: Studies on circulation. IV. Further analysis of the injection method and of changes in hemodynamics under physiological conditions. *Amer. J. Physiol.*, 1932, *99*, 534.

(3) HURTADO, A.: Respiratory adaptation in the Andean natives of the Peruvian Andes. Studies at high altitude. *Amer. J. of Physical Anthrop.*, 1932, *17*, 137.

(4) HURTADO, A., MERINO, C., DELGADO, E.: Influence of anoxemia on the hemopoietic activity. *Arch. intern. Med.*, 1945, *75*, 284.

(5) JASAHUI, E.: *Tesis de Bachillerato.* Fac. Med. Lima, 1954 (Not published).

(6) KINSMAN, J. M., MOORE, J. W., HAMILTON, W. F.: Studies on the Circulation. I. Infection method: Physical and mathematical considerations. *Amer. J. Physiol.*, 1929, *89*, 322.

(7) LEE PEARCE, M., MC KEEVER, W. P., DOW, P., NEWMAN, E. V.: The influence of injection site upon the form of dye dilution curves. *Circulation Res.*, 1953, *1*, 112.

(8) MONGE, C.: Biological basis of human behavior. *In Anthropology today.* The University of Chicago Press, 1953, 127-144.

(9) MOORE, J. W., KINSMAN, J. M., HAMILTON, W. F., SPURGLING, R. G.: Studies on the circulation. II. Cardiac output determinations; comparison of the injection method with the direct Fick procedure. *Amer. J. Physiol.*, 1929, *89*, 331.

(10) NEWMAN, E. V., MERREL, M., GENECIN, A., MONGE, C., MILNOR, W. R., MC KEEVER, W.: The dye dilution method for describing the central circulation. An analysis of factors shaping the time-concentration curves. *Circulation*, 1951, *4*, 735.

(11) ROTTA, ANDRÉS: *Sumario sobre el presente estado de las investigaciones en fisiología cardiovascular del hombre nativo que vive a 4500 metros de altitud.* Symposium on the Physiology of High Altitudes, Lima 1949.

(12) SCHREINER, G. E., FREINKEL, N., ATHENS, J. N., STONE, W.: Cardiac output central volume and dye injection curves in traumatic arteriovenous fistulas in man. *Circulation*, 1953, *7*, 718.

(13) STEWART, G. N.: The pulmonary circulation time, the quantity of blood in the lungs and the output of the heart. *Amer. J. Physiol.*, 1921, *58*, 20.

INFLUENCE OF SODIUM PHENYL-DIMETHYL-PYRAZOLONE-METHYL-AMINOMETHANE SULPHONATE (NOVALGIN, NEOMELUBRIN) ON THE CHICK EMBRYO

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THERE HAS BEEN recent interest in determining whether the beneficial effects obtained by the use of the analgetic-antipyretic group of drugs in patients with arthritic disorders is to be atributed to mechanisms other than the direct diminution of pain. Cortisone-like effects have been demonstrated for several members of the group. Thus, salicylic acid and derivatives have been found to produce adrenal ascorbic acid depletion ([1], [2]), presumably by stimulation of the pituitary gland ([3]). A drop in the cholesterol content of adrenal glands as a result of salicylamide administration to rats has also been reported ([2]). Likewise, aspirin ([4]) and phenylbutazone ([4], [5]) produce an inhibition of localy induced inflamatory reaction. Phenylbutazone has also been shown to produce sodium and water retention in animals ([6], [7]) and in man ([8]). However, these findings can not be generalized to other cortisone actions, nor can they be atributed to pituitary stimulation, at least in the case of pyrazolone derivatives, in view of the fact that there has been no observable increase in the excretion of 17-ketosteroids, nor depression in the number of circulating eosinophiles, nor any hyperglicemia after the administration of phenylbutazone ([7]). In the same token, sodium salicylate and aspirin have been found to produce a marked diminution in liver glycogen ([9]) a response in the opposite direction of that which results from the administration of cortisone.

It has, therefore, seemed convenient to investigate whether a member of the pyrazolone group shares, with adrenal steroids, their capacity for inhibition of growth, represented typically by their effect on developing chick embryos ([10], [11], [12]). Neomelubrin was choosen because of its ready aqueous solubility and because the results obtained with

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it may be more legitimatelly applied to other pyrazolone derivatives, in view of the facts it differs less radically than phenylbutazone from either member of the groups.

Methods

White Leghorn eggs of 6 or 8 days incubation were used. Sodium phenyl-dimethyl-pirazolone-methyl-aminomethane-sulphonate in aqueous solution or cortisone acetate suspended in saline, made up to a total volume of 0.1 ml, were injected via the chorioallantoic membrane using Beveridge and Burnet's technique ([12]). Controls received an equal volume of saline. Injected eggs were kept in the incubator at 37°C for 8 days, at the end of which period they were opened. Total wet body weight and dry body weight were measured. In some cases, the relative wet weight of the liver was estimated.

Results

The inhibition of body growth obtained in chick embryos injected with cortisone was of a similar magnitude of that reported by others ([11]). Interesting findings in this group of embryos, apart of the gross changes already reported, were frequent intraocular hemorrhages, epithelial vascular fragility, early invasion of the amniotic fluid by protein and a green discoloration of the liver. These phenomena are being investigated and the findings will be reported elsewhere.

TABLE I

Inhibition by Neomelubrin of the growth of the chick embryo

Dose mg/egg	Number	Age days	Weight grams	Inhibition percent	t	P
0.6	12	16	12.3 ± 1.4	9.5	3.0	< 0.01
2.5	40	16	11.8 ± 1.2	13.2	6.0	< 0.001
5.0	44	14	7.8 ± 0.6	15.2	7.5	< 0.001
10.0	24	16	11.1 ± 1.0	18.4	8.2	< 0.001
0.0	26	16	13.6 ± 1.2	—	—	—
0.0	44	14	9.2 ± 1.1	—	—	—

Neomelubrin injected eggs resembled normal controls more closely in gross appearance. However, there was a clear inhibition of growth. Table I summarizes the data pertinent to the evaluation of the magnitude of this effect. The table includes the number of eggs in each group, the mean wet body weight of experimental and control groups, the corresponding standard deviations, the percentage inhibition of growth obtained with each dose level and the estimates of statistial significance. The results of higher dose levels are not included because of high mortality. The last dose level reported —10 mg per egg— represents the approximate LD_{50} for the drug. Animals receiving 5 mg per embryo showed a mortality of 12 percent. In the groups marked 14 days as well as in those marked 16 days the drug had been administered 8 days prior to the opening of the eggs, that is, respectively, on the 6th and 8th days of incubation. Controls were included for both types.

The magnitude of the response was apparently a linear function

of the logarithm of the dose, as can be seen from figure 1 in which the percentage of inhibition of growth:

$$\frac{\text{Mean Wet Weight Normals} - \text{Mean Wet Weight Experimentals}}{\text{Mean Weight Normals}} \times 100$$

is plotted against the logarithm of the dose expressed in miligrams of neomelubrin per egg.

When the water content of control and Neomelubrin injected eggs was measured it was found that the mean percent water content of the

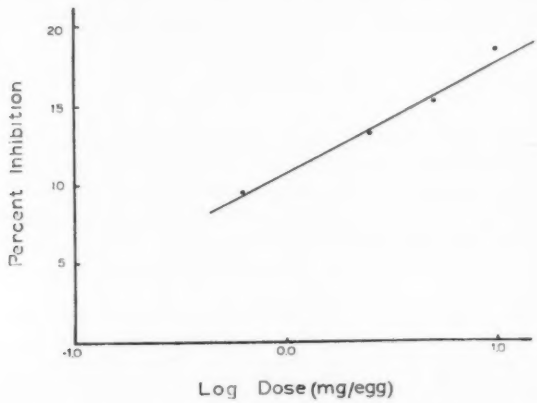

FIG. 1. — *Dose response curve for the influence of Neomelubrin on the growth of the chick embryo. The abscissae represent logarithm of the dose, expressed in mg per egg and the ordenates effect in percent diminution of wet body weight.*

former was 87.4 ± 1.2 and the latter 89.2 ± 0.6. This fact implies that, on the basis of dry weight, the difference in growth between the groups compared is slightly greater than that reported on the basis of total weight. These figures correspond to eggs receiving 5 mg of cortisone. In this group, the mean dry weight for controls was 1.16 g and for neomelubrin injected embryos 0.84 g.

The relative weight of the livers in the Neomelubrin injected group did not differ significantly from that in the control animals. The gross appearance of the livers in the former group was similar to that of the controls, in contrast to the changes referred to above in the appearance of the livers in the cortisone injected embryos.

DISCUSSION

It has been interesting to confirm a cortisone-like effect in a member of the pyrazolone group of analgetic-antipyretic drugs, providing further support to the hypothesis that the usefulness of these substances in rheumathic states is not limited to the abolition of pain and reflex

contractures. In what refers to inhibition of growth, Neomelubrin is apparently about 10 times less active than cortisone on a weight basis. This agrees approximately with the relative useful clinical doses and is of the same order as the difference reported by Selitto and Randall (4) when comparison is made between the substances in what refers to the inhibition of local inflamatory reaction. The increase in water content at the 5 mg level is only slightly lower than that reported by Evans (11) using 1 mg of cortisone. This shows a relative potency of the order already mentioned.

Further reasearch is neccessary before the results observed, and, for that matter, the growth inhibition due to the use of cortisone itself, can be adscribed to influence on specific metabolic proccesses and not to general toxic effects. Such studies are in progress in this laboratory.

Summary

The effects of sodium phenyl-dimethyl-pyrazolone-methyl-amino methanesulphonate (Neomelubrin) on the developing chick embryo has been studied. An inhibition of growth and an increase in water content, both similar to those obtained with cortisone, were observed. No effect was apparent in the relative size of the liver.

References

(1) Cronheim, G., King, J. S.: *J. Pharmacol.* 1951, *103*, 341.
(2) Forbes, J. C., Board, J. A., Duncan, G. M.: *Proc. Soc. exper. Biol.*, N. Y., 1954, *85*, 37.
(3) Cronheim, G., King, J. S. Jr., Hyder, N.: *Proc. Soc. exper. Biol.*, N. Y., 1952, *80*, 51.
(4) Selitto, J. J., Randall, L. O.: *Fed. Proc.*, 1954, *13*, 403.
(5) Domejoz, R.: *Fed. Proc.*, 1952, *11*, 339.
(6) Campbell, E. F.: *Proc. Soc. exper. Biol.* N. Y., 1953, *84*, 481.
(7) Chenkin, T., Brodie, B. B., Lee, P., Burns, J. J., Lowman, E. W., Goloman, A., Steele, M. A.: *Fed. Proc.*, 1953, *12*, 310.
(8) Green, J., Williams, P. O.: *Lancet*, 1953, *1*, 575.
(9) Comulada, G. M., Carló, P. E., Smith, P. K.: *Fed. Proc.*, 1953, *12*, 313.
(10) Landauer, W.: *Endocrinology*, 1947, *41*, 489.
(11) Karnofsky, D. A., Stock, C. C., Rhoads, C. P.: *Fed. Proc.*, 1950, *9*, 290.
(12) Evans, H. J.: *Proc. Soc. exper. Biol.* N. Y., 1953, *83*, 31.
(13) Beveridge, W. I. B., Burnet, F. M.: *Spec. Res. Series Med. Res. Counc.*, London, 1946, *14*, 256.

THE ACTION OF ESTRADIOL UPON BONE GROWTH "IN VITRO"

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THE ACTION of the gonadal hormones upon the skeletal development has been studied in animals. It is generally admitted that estrogens produce an inhibition of skeletal development. But it is not yet clear whether this is a direct influence upon the growth of cartilage and ossification, an indirect action through other endocrine glands, particularly the hypophysis, or a general alteration of metabolism, or a combined action.

The problem may be favorably faced by studying the action "in vitro" of steroids upon the growth of embryonic bones, with the addition of adequate concentrations of the hormone to the culture medium. In this way the possible indirect actions through other glands, that made the interpretation of the results difficult, are discarded.

MATERIAL AND METHODS

The femurs of chick embryos of 7 to 8 ½ days of incubation, dissected under the binocular microscope, are placed upon the surface of a clot of plasma contained in a watch glass in the moist camera of a Petri capsule (Fell & Robinson's method) (1). One femur of each embryo is explanted into a clot with estradiol, using the other one as a control. The clot is formed of 4 drops of chick plasma, 2 drops of embryonic extract diluted with Tyrode, and 2 drops of estradiol solution in Tyrode. Since estradiol * is diluted in NaOH 0.1 %, the controls have 2 drops of Tyrode plus the corresponding proportion of NaOH 0.1 %.

The concentration of estradiol in the total medium varies between 1 in 80 000 and 1 in 32 000 (an excess to produce saturation). The femurs are transplanted each 2 to 3 days. Inmediately after the initial explantation, and before each transplantation (or each 2 transplantations) the length of the femur is measured, using an ocular micrometer, expressing the size in arbitrary units equivalent to 0.16 mm.

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* We thank Dr. Benigno Anido of the "Dispert" Laboratories for the supply of the estradiol used in these experiments.

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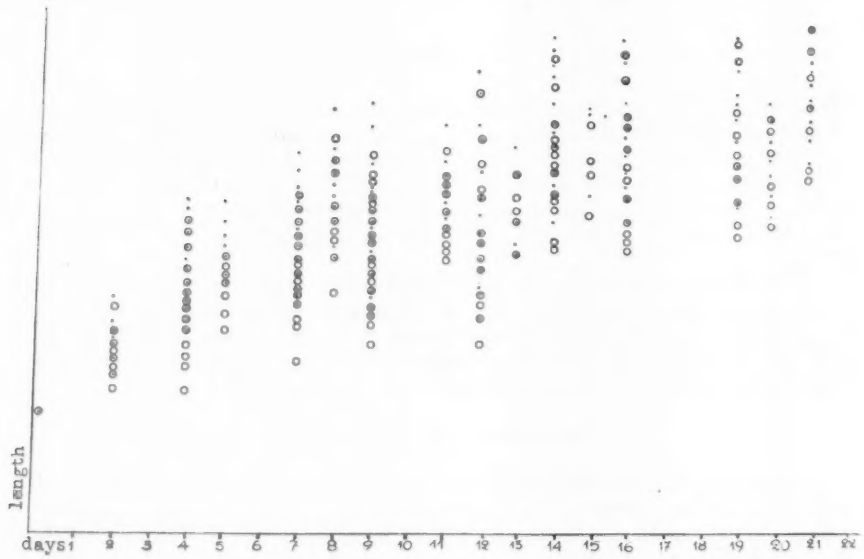
The total amount of experiments was 247, of which 63 were eliminated from the records because the treated bone or its control presented defects in its development (crookedness) or had been accidentally infected. The observations lasted in some series up to 27 days (growth up to 21); in others to a variable number of days, with a minimum of 8.

Results

With low concentrations (1 in 800 000 to 1 in 400 000) no action favourable or inhibitory was observed in the growth curves. Progressively higher concentrations were used, reaching concentrations beyond the so-



Graph of the increases in length of 56 femurs treated with estradiol 1/52000 (points) and their controls (circles). The initial sizes are supposed to be equal.

lubility of estradiol in the medium, up to 1 in 32 000. With this saturation, several series of experiments were registered in which the bones treated with estradiol grew more than the controls, though moderately. This increase in growth was more marked during the first days of development "in vitro", and was observed in different degrees 40 times in 56 (71.4 %), always moderately.

It may be stated that estradiol "in vitro" has no inhibitory action upon the growth of the embryonic bone. The stimulating action observed with high concentrations is easy to see in fig 1, and the statistical

* We thank Dr. W. De Angelis for realizing this study.

study * shows that in 8 days embryos, the action is significant in the first 5 days of culture, being non significant from the 8th day on.

GROWTH OF THE FEMUR OF AN 8 DAY EMBRYO

Normal and treated with estradiol (concentration 1/32000)

	4 days		*5 days*		*8 days*	
	Control	*Treated*	*Control*	*Treated*	*Control*	*Treated*
Nº	19	19	16	16	29	29
M	6.24	7.11	6.70	7.81	7.94	8.59
ε	0.25	0.19	0.26	0.19	0.36	0.36
Difference between averages	+ 0.87		+ 1.11		+ 0.65	
P	< 0.01		< 0.01		N.S.	

Nº Number of femurs studied.
M Arithmetic average of increase in length.
ε Standard Error of the Average or Probable Error.
P Probability calculated from the table of values of t.

* Expressed in arbitrary units : 1 mm = 6.24.

Discussion

Based on many experiments with castrated animals treated by different ways it is generally admitted that estrogens produce a diminution of growth.

Castrated rats before puberty present no difference from the controls, until these reach the puberal age, when the ovaries start to produce estrogens. But if they are castrated just after puberty, it is easily seen that castrated animals grow more ([2]).

The conjunction cartilages are thickened, principally the columnal cartilage. The process of ossification is slower than in the intact animal ([3], [4]).

Contrarywise the administration of estrogens produces decrease in height, thinness of the cartilages, increase of the subepiphysial osseous spiculae and precocious soldering of the epiphysis by a more active ossification ([5], [6], [7], [8], [9]).

Therefore, an inhibitory action of the estrogens upon the proliferation of the cartilage exists, which may be neutralized by administration of pituitary growth hormone ([7]). In order to know whether this action is mediated through the hypophysis or not, the ovaries and hypophysis of young rats were removed: 5 days later, one group was grafted with hypophysis of normal animals, another group was grafted with hypophysis of animals previously treated with estradiol. The action upon growth was smaller in this last group, indicating an indirect action through the hypophysis ([10]).

On the other side, the difference obtained in relation between tail growth and body weight in hypophysectomized animals, and in animals first treated with estilbestrol and afterwards with pituitary extract, shows that estrogens do not act solely through the hypophysis.

Finally the subespiphysial alterations in hypophysectomized rats

and in those treated with estrogens are histologically different, indicating that the estrogens not only act by inhibiting the hypophysis but may act directly ([7]).

Our experiments show not only that estradiol acting "in vitro" has no inhibitory action upon the chick's embryonic femur, but has at high doses, a slight stimulating action, although of a short duration (5 first days of culture). These results are fundamentally different from those obtained by other authors in animals. According to them moderate doses of estrogens have no effect, high doses produce an inhibition in relation to the dose (up to a limit) and may stop growth; while other authors have found that low doses have a stimulating action ([12]).

The highest doses "in vitro" (saturation of the culture medium) produced a slight stimulation. This concentration is probably never reached in the animal's tissues, even if intensely treated, and furthermore one should not compare the effects of equal concentrations "in vitro" and "in vivo". But as in the intact animals the inhibitory action increases with concentration, it must be supposed that if saturation is reached, the inhibitory effect would be maximal and not stimulating

The bones used in these experiments are embryonic, of a few days of incubation and of cartilaginous structure. It seems improbable that its reactions to hormones may be different from those characteristic of a developing bone, after birth.

The absence of an inhibitory effect "in vitro" upon the embryonic material leads us to suggest that the growth-inhibiting action of estradiol "in vivo" is indirect through the hypophysis or through some other mechanism.

SUMMARY

The method of culture "in vitro" permits the complete dissociation of the direct hormonal action upon the effector, from the indirect action. Our results show that estradiol has no direct inhibitory influence; on the contrary, a slight stimulating action on bone growth (chick embryo's femur) was found.

BIBLIOGRAPHY

(1) FELL, H. B., ROBINSON, R.: *Biochem. J.*, 1929, *23*, 767.
(2) BOGART, R., LASLEY, J. F., MAYER, D. T.: *Endocrinology*, 1944, *35*, 173.
(3) SILBERBERG, M., SILBERBERG, R.: *Amer. J. Path.*, 1939, *15*, 55.
(4) SILBERBERG, M., SILBERBERG, R.: *Amer. J. Path.*, 1940, *16*, 491
(5) SPENCER, J., D'AMOUR, F. E. GUSTAVSON, R. G.: *Amer. J. Anat.*, 1932, *50*, 129.
(6) ZONDEK, B.: *Lancet*, 1936, *2*, 842.
(7) GAARENSTROOM, J. H., LEVIE, L. H.: *J. Endocrinol.*, 1939, *1*, 420.
(8) DAY, H. G., FOLLIS, R. H.: *Endocrinology*, 1941, *28*, 83.
(9) SIMPSON, M. E., KIBRICK, E. A., BECKS, H., EVANS, H. M.: *Endocrinology*, 1942, *30*, 286.
(10) REECE, R. P., LEONARD, S. L.: *Proc. Soc. Exper. Biol. & Med.*, 1939, *42*, 200.
(11) GRIFFITHS, M., YOUNG, F. G.: *J. Endocrinol.*, 1942, *3*, 96.
(12) LICHWITZ, A., THIÉRY, G., PARLIER, R., DELAVILLE, M.: *Semaine d. hop. Paris*, 1951, *27*, 247.

EFFECT OF HYPOPHYSECTOMY IN THE TURTLE "CHRYSEMYS D'ORBIGNYI"

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THE EFFECTS of hypophysectomy have been studied in different animal species. The present work has been carried out with the purpose of investigating the effect of hypophysectomy in the turtle "Chrysemys d'Orbignyi" * and to widen the knowledge of hypophyseal functions in chelonians.

OPERATIVE TECHNIQUE

The animal was anesthetized by being put into a small closed chamber containing 20 to 30 ml of ether and left here 30 to 40 minutes. It was then placed on its back and its limbs and head were fixed to the table with its mouth wide open. The animal was placed so that the head end was towards the operator.

Hypophysectomy was performed by the transpalatine approach, making a vertical incision in the middle of the palate of approximately 2.5 cm long, starting at 0.5 cm from the nostrils. In the rostral end of this incision, another one of 1.5 cm was made, perpendicular to the first. After lifting up the periosteum, trephination was performed in the center of the triangle of exposed bone, using a dental drill. The shape of the hole in the bone should be eliptical and 6 x 4 mm, with its greater axis lying in the midline of the animal.

Once the bone has been perforated, the hypophysis is easily seen and removed by means of a pipette connected to a vacuum pump. A dissecting needle is used to destroy meningeal tissue and hypophyseal connections. This technique has been previously published in detail (Wagner, 1955).

MATERIAL AND METHODS

Seventy-six female turtles, weighing 1.40 to 2.86 kg and 13 males weighing 1.18 to 1.88 kg were operated on. A group of 48 turtles of

* As classified by the National Museum of Río de Janeiro.

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both sexes were used as normal controls. The tissues for the histological study were fixed in formol, Bouin or Helly and stained with hematoxylin-eosin, Mallory-Azan, Gomori, MacManus or Sudan Black stains.

RESULTS

Feeding. Both normal and hypophysectomized animals were fed dead fish "ad libitum". Apparently they fasted during winter and autumn. The hypophysectomized turtles seemed to eat a larger quantity of fish than the normals did.

TABLE I

Changes in body weight of normal and hypophysectomized female turtles

	Nº of animals	Body weight (mean)	
		Initial	6 months after operation
Normal	6	2 096 g	2 104 g
Hypophysectomized	22	1 907 g	1 867 g

Survival. Only 4 animals out of 89 operated died of surgical accidents. In each case, a large hemorrhage had been always observed during the operation. Survival after hypophysectomy was markedly lower in males than in females. During a period of two months 50 % of the males died while during the same period only 5 % of the females died. The survival time in some animals was rather long: 4 males were maintained 8 to 11 months and 4 females 15 to 16 months after the operation.

Body weight. Six adult normal and 22 hypophysectomized turtles were weighed during 6 months. No significante variations were observed as can be seen in table I.

Weight of Organs. Different organs of 25 normal and 16 hypophysectomized females were weighed. Significant differences were found in the adrenals, ovaries, oviduct and thymus whereas there were no differences observed in the thyroid, pancreas, spleen and shell. These results are presented in table II.

Sexual behaviour. During the spring, sexual relations were observed in normal turtles but not in the hypophysectomized animals. Autopsy revealed calcified eggs in the oviduct of 33 % of the normal females, during spring and summer. This was never observed in those hypophysectomized. However, two females operated in summer, released incompletely calcified eggs after hypophysectomy.

Glycemia. Eighty-six females were studied (43 hypophysectomized and 43 normal). Blood was collected from the jugular vein after 24 hours

TABLE II

*Weight of organs of normal and hypophysectomized female turtles, sacrificed 80 ± 6 * days after operation*

	Normal	Hypophys-ectomized	P **
Nº of animals	25	16	
Body weight (g)	1 783 ± 53 *	1 873 ± 19	> 0.1
Thyroid (mg)	355 ± 31	364 ± 46	> 0.5
Adrenals (mg)	158 ± 9	121 ± 4	< 0.01
Ovaries (g)	85 ± 5	52 ± 6	< 0.01
Oviducts (g)	33 ± 1	25 ± 1	< 0.01
Thymus (mg)	297 ± 32	184 ± 20	< 0.01
Pancreas (mg)	2986 ± 137	2799 ± 116	> 0.3
Spleen (mg)	2871 ± 147	2874 ± 161	> 0.9
Shell (g)	565 ± 18	627 ± 32 ***	> 0.05

* Mean ± standard error.

** Probability.

*** 8 animals.

fasting. It was observed that the annual and seasonal average of blood sugar was 50 % lower in hypophysectomized animals than in the normals. The differences were markedly significant (see table III).

Glycogen. Liver, muscular and cardiac glycogen was determined in 18 normal and 14 hypophysectomized females. Before glycogen determinations, all the animals were given 1 ml/kg of Intocostrine-T (Squibb) intramuscularly. After 30 to 40 minutes, they were decapitated and the samples analyzed according to Pflüger's method.

In a study of 12 normal and 8 hypophysectomized females after 24 hours fasting, a significant decrease in glycogen was observed in the latter (liver 69 % and muscular 59 %). A slighter difference was observed in cardiac glycogen (23 %) but was not statistically significant (Table IV).

TABLE III

Seasonal and annual changes of glycemia in normal and hypophysectomized female turtles, after 24 hours fasting

		Normal	Hypophys-ectomized	P **
Summer (21° C to 31° C) ***	Nº of animal	21	8	—
	Days after operation	—	49 ± 16 *	—
	Glycemia (mg %)	88 ± 5	53 ± 8	< 0.01
Autumn (13° C to 22° C)	Nº of animal	7	12	—
	Days after operation	—	62 ± 12	—
	Glycemia (mg %)	95 ± 10	42 ± 7	< 0.01
Winter (11° C to 20° C)	Nº of animal	8	17	—
	Days after operation	—	76 ± 7	—
	Glycemia (mg %)	71 ± 12	32 ± 3	< 0.01
Spring (17° C to 27° C)	Nº of animal	7	6	—
	Days after operation	—	65 ± 8	—
	Glycemia (mg %)	96 ± 14	57 ± 4	< 0.02
Annual (16° C to 25° C)	Nº of animal	43	43	—
	Days after operation	—	65 ± 5	—
	Glycemia (mg %)	87 ± 5	42 ± 3	< 0.01

* Mean ± standard error.

** Probability.

*** Mean temperatures, minimal and maximal.

TABLE IV

Glycogen and blood sugar changes in normal and hypophysectomized female turtles, after 24 hours fasting

		Normal	Hypophys. ectomized	P **
Number of animals		12	8	—
Days after operation		—	87 ± 9 *	—
Glycemia (mg %. g)		86 ± 8	37 ± 9	< 0.01
Glycogen (mg % g)	Liver	7 219 ± 677	2 283 ± 427	< 0.01
	Muscle	1 597 ± 184	665 ± 166	< 0.01
	Heart	1 149 ± 110	892 ± 234	> 0.3

* Mean ± standard error.
** Probability.

A glucose solution was administered *per os* to another group of 6 normal and 6 hypophysectomized females, after 24 hours fasting. Each animal was given 500 mg/kg of glucose and the glycogen was determined 6 hours later. It was observed that there was no difference between the normals and the hypophysectomized in the elevation of the blood glucose following the meal. In normals, the glycogen was not increased whereas that in the hypophysectomized was higher than that after fasting (Table V).

Histological changes

Thyroid. The weight of the thyroid was not changed probably due to there being no changes in the amount of the colloid in the follicles. However, there was pronounced atrophy of the follicular cells which were cubical instead of columnar as seen in the normal turtle. The nuclei were also smaller, more compact and fusiform in contrast to spherical shape in the normal. The atrophy of the cytoplasm was greater than that of the nucleus, thus the relation between the cellular height and the diameter of the nucleus was 1.5 to 2.5 instead of 3.0 to 3.5 as in the normal. The colloid adjacent to the cells appear less vacuolated in the hypophysectomized than in the normal (fig 1 and 2).

Adrenal. The adrenal cortical cells in the hypophysectomized animal were markedly atrophied and contained large vacuoles which sometimes nearly occupied all the cell. The nuclei lost their normal spherical shape and the chromatin was condensed; at times, pyknotic nuclei were observed. In the adrenal of the hypophysectomized turtles there were a larger

TABLE V

Glycogen and blood sugar changes in normal and hypophysectomized female turtles, after 24 hours fasting and 6 hours ofter oral glucose administration (500 mg/kg)

		Normal	Hypophys. ectomized	P **
Number of animals		6	6	—
Days after operation		—	89 ± 14 *	—
Glycemia (mg %)	Fasting	84 ± 6	49 ± 6	< 0.01
	6 hours after glucose	121 ± 17	67 ± 10	< 0.02
Glycogen (mg % g)	Liver	5 923 ± 466	3 372 ± 715	< 0.02
	Muscle	1 402 ± 256	928 ± 240	> 0.1
	Heart	1 646 ± 287	1 541 ± 170	> 0.7

* Mean ± standard error.
** Probability.

number of focci of hemopoiesis and decrease in the number of granulated eosinophils of the type described by Stilling in the batrachians (Fig 3 and 4).

Ovaries. In the hypophysectomized turtles, there were regressive changes in the follicles and in the ovocytes. In the former, there was moderate atrophy of the follicular epithelium and of the muscular fibers of the internal wall. In the ovocytes, the protoplasm contained many vacuoles in the peripheral zone. In the more mature ovocytes, the germinated vessicle was disorganized and, in some cases, was not present.

Oviduct. The epithelium in the oviduct of hypophysectomized turtles was atrophic with decrease in the height of the cells and with alterations in the structure of the nuclei. The cilia were also shorter. Both the tubules and the acinii of the oviductal glands were markedly atrophic. There was a great diminution in the secretion granules of the glandular cells and in some tubules there was a complete absence of the granules (fig 5, 6, 7 and 8).

Cloacal epithelium. In the hypophysectomized there was atrophy of the mucosa of the cloaca with a decrease in the cellular height and in the mucin content (fig. 9 and 10).

Testes. In the testes of the hypophysectomized turtles, the diameter of the seminiferous tubules was decreased. The number of spermatocytes

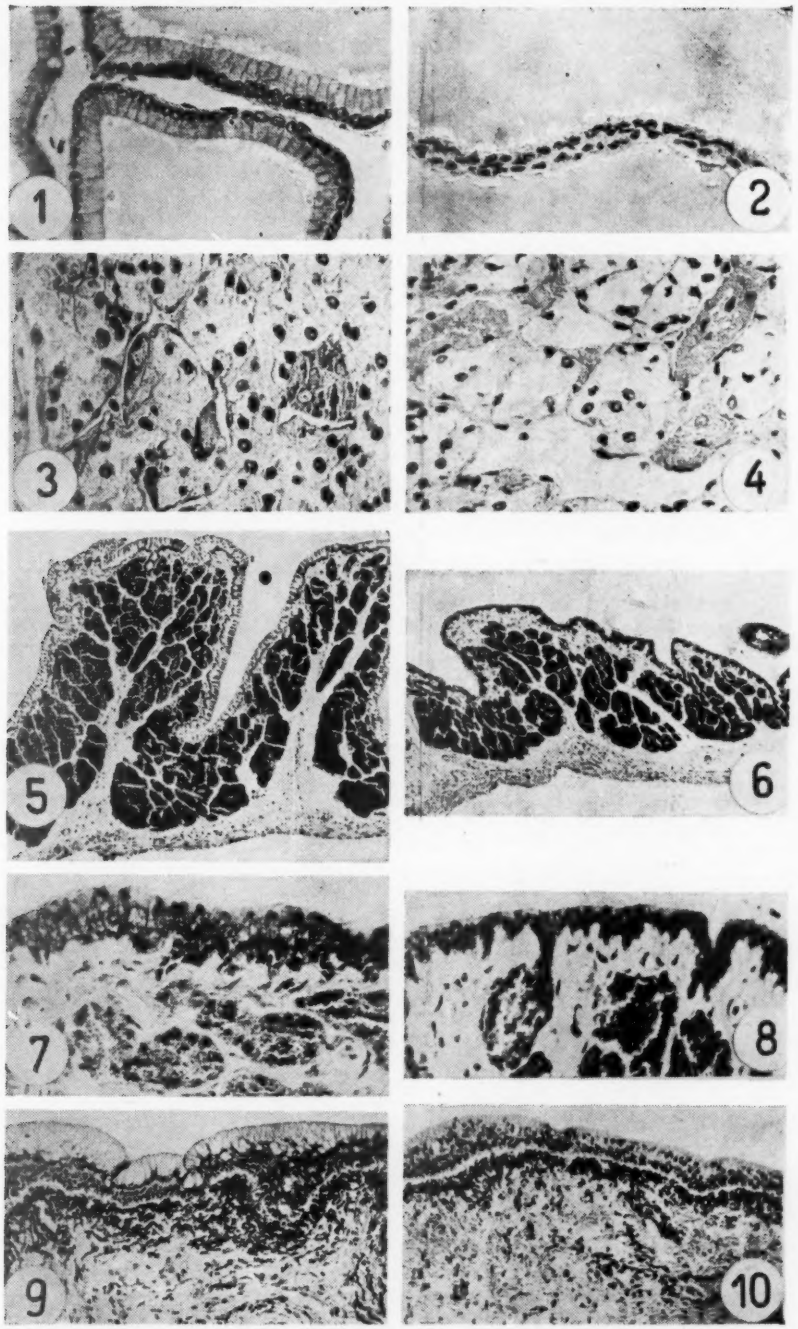

FIG. 1. — *Thyroid, normal (H. E. × 480). Fig. 2. Thyroid, hypophysectomized (H. E. × 480). Fig. 3. Adrenal, normal (H. E. × 520). Fig. 4. Adrenal, hypophysectomized. (H. E. × 520). Fig. 5. Oviduct, normal (H. E. × 120). Fig. 6. Oviduct, hypophysectomized (H. E. × 120). Fig. 7. Oviduct, normal (Pas × 480). Fig. 8. Oviduct, hypophysectomized (Pas × 120) Fig. 9. Cloacal epithelium, normal (H. E. × 240). Fig. 10. Cloacal epithelium, hypophysectomized (H. E. × 240).*

in the lumen of the tubule was diminished and at times completely absent. Spermatogenesis was markedly arrested generally at the stage of the primary and secondary spermatocytes and there were immature cells frec in the tubular lumen (fig 11 and 12).

In one animal there was a complete absence of all germinative cells; there being only Sertoli cells present. The cytoplasm of the Leydig cells in the hypophysectomized animals had a larger number of vacuoles.

Thymus. The lobules of the thymus were smaller in the hypophysectomized animals. There was evidence of involution of the thymocytes and proliferation of the fibroblastic elements.

Skin. The skin of the neck of normal and hypophysectomized animals was studied. In the latter, there was atrophy of the epidermis with a marked decrease in the number of cells of the basal layer and the spinous layer (fig. 13 and 14).

The pigmented cells of the dermis were smaller in the hypophysectomized than in the normals.

Liver. The normal arrangement of the hepatic cells in cords was not observed in the hypophysectomized animals. The hepatocytes had greatly increased in size and demonstrated intracellular changes. With hematoxylin-eosin stain, the cytoplasm was occupied by a large vacuole which displaced the nucleus from its normal central position. These vacuoles which could be intensely stained by Sudan Black B were much larger and more numerous than the granules similarly stained in the normals. The nuclei were very deformed and many were markedly pyknotic. In the section stained with PAS (McManu) the glycogen content of the liver cells was greatly diminished in the hypophysectomized animals (fig 15 and 16).

Spleen. The granular eosinophilic cells which occupied the venous sinus of the spleen were greatly decreased in number in the hypophysectomized animal. The lymphoid follicles also were much smaller than in normals (fig 17 and 18).

Heart, kidney and pancreas. No significant histological alterations were observed in our preparations.

Discussion

The turtle "Chrysemys d'Orbignyi" survived a long time after hypophysectomy and was in good general condition. Females survived as long as 15 to 16 months and males, 8 to 11 months. The sexual difference deserves further study.

In the turtle as in other species, atrophy, both macro and microscopical, ofter hypophysectomy was observed in certain endocrine glands especially the ovary, testicle and adrenals. There was no decrease in the weight of the thyroid, probably due to lack of reabsorption of the colloid, but histological study showed a marked atrophy of the follicular epithelium.

Marked atrophy of the skin, cloacal epithelium, oviduct and liver was also observed.

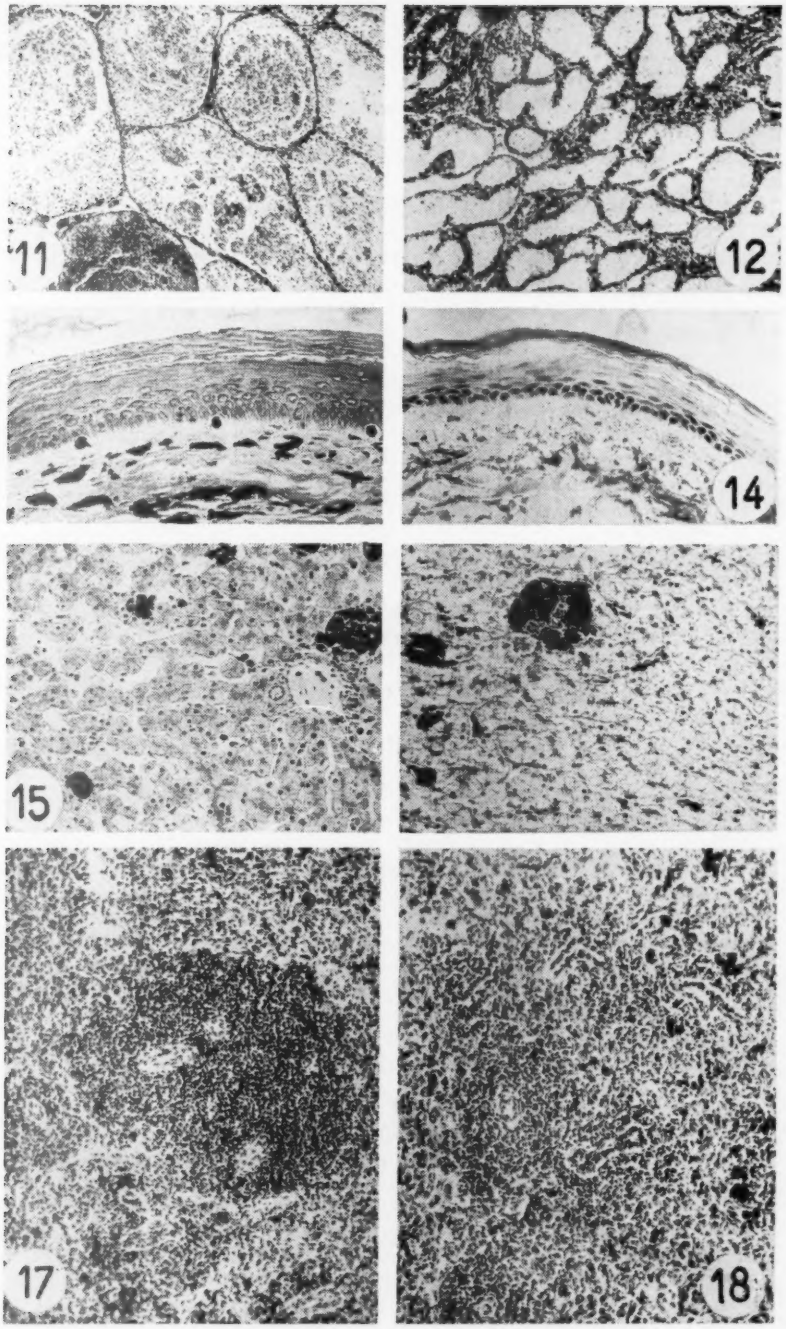

FIG. 11. — *Testicle, normal (H. E. × 120). Fig. 12. Testicle, hypophysectomized (H. E. × 120). Fig. 13. Skin, normal (H. E. × 360). Fig. 14. Skin, hypophysectomized (H. E. × 360). Fig. 15. Liver, normal (H. E. × 160). Fig. 16. Liver, hypophysectomized (H. E. × 160). Fig. 17. Spleen, normal (H. E. × 120). Fig. 18. Spleen, hypophysectomized (H. E. × 120).*

No changes were found in the weight of the body, shell, pancreas and spleen.

There were no calcified eggs in the oviduct as observed in the normal animals.

The metabolic alterations in the hypophysectomized turtle were pronounced. In normal animals, glycemia decreases in winter, rises in spring, somewhat decreasing in summer and rises again in autumn. On the contrary, in the hypophysectomized, glycemia was lower in autumn than in summer. Seasonal variations were similar although the fasting glycemia was significantly lower in the hypophysectomized than in normals.

Liver, muscular and cardiac glycogen were lower in the hypophysectomized turtles both after fasting or after oral glucose administration.

As proviously demonstrated (Lopes, Wagner, Barros and Marques, 1954) the rise in blood glucose after the administration of glucose or adrenaline was less in the hypophysectomized turtles whereas the fall after insulin administration was greater than that in the normal. The rise in the blood glucose level after pancreatectomy was less in hypophysectomized than in normals (Foglia, Wagner, Barros and Marques, 1955).

These observations demonstrate that the disturbances in carbohydrate metabolism occurring in the turtle following hypophysectomy are similar to those observed in other species.

When hypophysectomy was incomplete, no atrophy of the endocrine organs was seen; in some cases only a partial involution.

Summary

Total hypophysectomy was performed in 89 turtles "Chrysemys d'Orbignyi" some of which were observed 8 to 16 months after the operation. The results were compared with those obtained in 48 controls.

1) the survival was longer in females than in males following hypophysectomy.

2) macro and microscopical atrophy of certain endocrine organs were observed in the hypophysectomized animals (adrenals, ovaries, testes).

3) microscopical atrophy was observed in the thyroid, oviduct glands, oviduct epithelium and cloacal epithelium and fatty infiltration in the liver.

4) the level of glycemia and of glycogen in the liver, muscle and heart was lower in the hypophysectomized turtles.

I express my acknowledgement to Prof. B. A. Houssay for his scientific advice; to Dr. A. F. Cardeza for his kind cooperation in carrying out the histological study and to the Rockefeller Foundation and Conselho Nacional de Pesquizas of Brasil for their support under which these investigations were done.

Bibliography

(1) Lopes, N., Wagner, E. M., Barros, M., Marques, M.: *Acta Physiol. latinoamer.*, 1954, *4*, 190.

(2) Foglia, V. G., Wagner, E. M., Barros, M., Marques, M.: *Rev. Soc. argent. Biol.*, 1955, *31*, 87.

(3) Wagner, E. M.: *An. Fac. Med. P. Alegre*, 1955, *15* (in press).

INDICE

AUTORES

ABELARDI, V.: Ver Villamil, A. y Col. 104
ALLENDE, I. L. C. de, Orías, O.: Hypophysis and ovulation in the toad **Bufo arenarum** Hensel 57
BARRIO, A. (h.): Spastic action of the venom of the spider **Phoneutria fera** 132
BITTNER, J. J.: Ver Martínez, C. y Col. 144
BRAUN MENÉNDEZ, E.: Professor Oscar Orías (1905-1955) 53
BUZZI, R. M.: Ver Villamil, A. y Col. 104
CAZORLA, A.: Ver Monge, C. y Col. 198
CLAVIJO, J.: Ver Villamil, A. y Col. 104
FERNANDEZ N., J.: Ver Whittembury M., G. y Col 117
FRANCO, R. J.: Ver Villamil, A. y Col. 104
GARCIA RAMOS, J.: Effects of radioactive phosphate on the excitability of mammalian nerves 85
GOMEZ POUYOU, A.: Ver Pardo, E. G. y Col. 211
GOYENA, H.: The action of estradiol upon bone growth "in vitro" 215
GUNTHER, B., Guerra, E.: Biological similarities 169
GUERRA, E.: Ver Günther, B. y Col. 169
GUTIÉRREZ VILLEGAS, L.: Ver Laguna, J. y Col. 155
HUIDOBRO, F.: Effects of decamethonium (C-10) on the neuro-muscular junction of soleus in the cat 1
HUIDOBRO, F.: On the mechanism of action of bis-(8'quinoliloxy) 1,5-pentane di-iodo ethylate (3381 RP) on the neuro-muscular junction 46
LAGUNA, J., Rodríguez, J., Gutiérrez Villegas, L., Pardo, E.: Effects of anoxia on the perfused cat liver 155
LAGUNA, J.: Ver Pardo, E. G. y Col. 211
LOPES, N.: The action of alloxan in the turtle Pseudemys d'Orbignyi D and B 39
MAGAN, L.: Ver Monge, C. y Col. 198
MARTINEZ, C., Miroff, G., Bittner, J. J.: The tumor size as a factor influencing the transplantability and lethality properties of mammary tumors in mice 144
MAZZELLA, H.: Aspects of hemodynamics of the carotid sinus in mammals 31
MIROFF, G.: Ver Martínez, C. y Col. 144
MONGE, C.: Ver Whittembury M., G. y Col. .. 117
MONGE, C., Cazorla, A., Whittembury, G., Sakata, Y., Rizo-Patrón, C., Magán, L.: A description of the circulatory dynamics in the heart and lungs of people at sea level and at high altitude by means of the dye dilution technique 198
ONDARZA, R.: Ver Pardo, E. G. y Col. 211
ORÍAS, O.: Ver Allende, I. L. C. de y Col. . 57
PARDO, E. G.: Ver Laguna, J. y Col. 155
PARDO, E. G., Gómez Pouyou, A., Ondarza, R., Laguna, J.: Influence of sodium phenyldimethyl-pyrazolone-methyl-aminomethane sulphonate (novalgin, neomelubrin) on the chick embryo 211
PENHOS, J. C.: Relationship between folic acid and sexual hormones in toads and rats 22
RAMIREZ V., M.: Ver Whittembury, G. y Col. 117
RIZO-PATRON, C.: Ver Monge, C. y Col. 198
RODRIGUEZ, J.: Ver Laguna, J. y Col. 155
RUSSEK, M.: Conditioning of tachycardia produced by histotoxic anoxia 122
RUSSEK, M.: Action of histotoxic anoxia on the salivary and cardiorespiratory conditional reflexes 187
SAKATA, Y.: Ver Monge, C. y Col. 198
SILVA, L.: Ver Vargas, L. y Col. 10
VARGAS, L., Silva, L.: On the problem of the absorption of protamine-zinc-insulin implanted subcutaneously. Presence of blood vessels in the capsule 10
VIAL, J. D.: The influence of axon length on the course of Wallerian degeneration of the motor end-plates 95
VILLAMIL, A., Clavijo, J., Franco, R. J., Buzzi, R. M., Abelardi, V.: Circulatory function in artificial hibernation 104
WAGNER, E. M.: Effect of hypophysectomy in the turtle "Chrysemys d'Orbignyi" 219
WHITTEMBURY M., G., Ramírez V., M., Fernández N., J., Monge C., C.: A nomographic system for calculation of water requirements 117
WHITTEMBURY, G.: Ver Monge, C. y Col. ... 198

MATERIAS

ALLOXAN, The action of, in the turtle "Pseudemys d'Orbignyi" D. and B. - N. Lopes 39
ANOXIA. Conditioning of tachycardia produced by histotoxic - M. Russek 122
ANOXIA, Effects of, on the perfused cat liver - J. Laguna y Col. 155
ANOXIA, Action of histotoxic, on the salivary and cardiorespiratory conditional reflexes - M. Russek 187
AXON, The influence of, length on the course of Wallerian degeneration of the motor end-plates - J. D. Vial 95
BIOLOGICAL SIMILARITIES. B. Günther y Col. 169
BONE GROWTH, The action of estradiol upon, "in vitro" - .H Goyena 215
CARDIORESPIRATORY, Action of histotoxic anoxia on the salivary and conditional reflexes - M. Russek 187
CAROTID SINUS, Aspects of hemodynamics of the, in mammals - H. Mazzella 31
CIRCULATORY function in artificial hibernation - A. Villamil y Col. 104
CIRCULATORY DYNAMICS, A description of the, in the heart and lungs of people at sea-level and at high altitude by means of the dye dilution technique - C. Monge y Col. 198
CHICK EMBRYO. Influence of sodium phenyldimethyl-pyrazolone-methyl-aminomethane sulphonate (novalgin, neomelubrin) on the - E. G. Pardo y Col. 211
DECAMETHONIUM, Effects of, (C-10) on the neuro-muscular junction of soleus in the cat - F. Huidobro 1
DYE DILUTION TECHNIQUE. A description of the circulatory dynamics in the heart and lungs of people at sea-level and at high altitude by means of the - C. Monge y Col. 198
END-PLATES. The influence of axon length on the course of Wallerian degeneration of the motor - J. D. Vial 95
ESTRADIOL, The action of, upon bone growth "in vitro" - H. Goyena 215
FOLIC ACID, Relationship between, and sexual hormones in toads and rats - J. C. Penhos . 22
HIBERNATION. Circulatory function in artificial - A. Villamil y Col. 104

HIGH ALTITUDE, A description of the circulatory dynamics in the heart and lungs of people at sea-level and at, by means of the dye dilution technique - C. Monge y Col. .. 198
HYPOPHYSECTOMY, Effect of, in the turtle "Chrysemys d'Orbignyi" - E. M. Wagner ... 219
HYPOPHYSIS and ovulation in the toad **Bufo arenarum** (Hensel) - I. L. C. de Allende y Col. 57
INSULIN, On the problem of the absorption of protamine-zinc-, implanted subcutaneously. Presence of blood vessels in the capsule - L. Vargas y Col. 10
LETHALITY PROPERTIES, The tumor size as a factor influencing the transplantability and, of mammary tumors in mice - C. Martínez y Col. 144
LIVER. Effects of anoxia on the perfused cat - J. Laguna y Col. 155
NEOMELUBRIN) on the chick embryo. Influence of sodium phenyl-dimethyl-pyrazolone-methyl-aminomethane sulphonate (novalgin, - E. G. Pardo y Col. 211
NERVES. Effects of radioactive phosphate on the excitability of mammalian - J. García Ramos 85
NEURO-MUSCULAR JUNCTION, Effects of decamethonium (C-10), on the, of soleus in the cat - F. Huidobro 1
NEURO-MUSCULAR JUNCTION. On the mechanism of action of bis(8'quinoliloxy) 1,5-pentane di-iodo ethylate (3381 RP) on the - F. Huidobro 46
(NOVALGIN, neomelubrin) on the chick embryo. Influence of sodium phenyl-dimethyl-pyrazolone-methyl-aminomethane sulphonate - E. G. Pardo y Col. 211
ORIAS, Professor Oscar (1905-1955) - E. Braun Menéndez 53
OVULATION, Hypophysis and, in the toad **Bufo arenarum** (Hensel) - I. L. C. de Allende y Col. 57
PROCEEDINGS of the Argentine Society of Biology 48, 114, 163
QUINOLILOXY, bis-(8'-, 1,5-pentane di-iodo ethylate (3381 RP) on the neuro-muscular junction. On the mechanism of action of - F. Huidobro 46
RADIOACTIVE PHOSPHATE. Effects of, on the excitability of mammalian nerves - J. García Ramos 85
REFLEXES. Action of histotoxic anoxia on the salivary and cardiorespiratory conditional - M. Russek 187
SALIVARY, Action of histotoxic anoxia on the, and cardiorespiratory conditioned reflexes - M. Russek 187
SEA-LEVEL, A description of the circulatory dynamics in the heart and lungs of people at, and at high altitude by means of the dye dilution technique - C. Monge y Col. 198
SEXUAL HORMONES, Relationship between folic acid and, in toads and rats - J. C. Penhos 22
SIMILARITIES. Biological. B. Günther y Col. .. 169
SPASTIC action of the venom of the spider **Phoneutria fera** - A. Barrio (h.) 132
TACHYCARDIA, Conditioning of, produced by histotoxic anoxia - M. Russek 122
TRANSPLANTABILITY, The tumor size as a factor influencing the, and lethality properties of mammary tumors in mice - C. Martínez y Col. 144
TUMOR SIZE, The, as a factor influencing the transplantability and lethality properties of mammary tumors in mice - C. Martínez y Col. 144
VENOM, Spastic action of the, of the spider **Phoneutria fera** - A. Barrio (h.) 132
WALLERIAN DEGENERATION, The influence of axon length on the course of, of the motor end-plates - J. D. Vial 95
WATER REQUIREMENTS. A nomographic system for calculation of - G. Whittembury y Col. 117

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centímetro	cm	centímetro cúbico	cm^3	gama	γ
milímetro	mm	mililitro	ml	por ciento	%
micrón	µ	kilogramo	kg	hora	h
milimicrón	mµ	gramo	g	minuto	m
Ångström	Å	miligramo	mg	segundo	s
				milisegundo	ms

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SUMARIO

Biological similarities. B. Günther and E. Guerra 169

Action of histotoxic anoxia on the salivary and cardiorespiratory conditional reflexes. Mauricio Russek 187

A description of the circulatory dynamics in the heart and lungs of people at sea level and at high altitude by means of the dye dilution technique. Carlos Monge C., Alberto Cazorla T., Guillermo Whittembury M., Yolanda Sakata B. and Carlos Rizo-Patrón. With the technical assistance of Luisa Magán S. 198

Influence of sodium phenyl-dimethyl-pyrazolone-methyl-amino-methane sulphonate (novalgin, neomelubrin) on the chick embryo. Efraín G. Pardo, Armando Gómez Pouyou, Raúl Ondarza and José Laguna 211

The action of estradiol upon bone growth "in vitro". H. Goyena ... 215

Effect of hypophysectomy in the turtle "Chrysemys d'Orbignyi". Edgar M. Wagner 219

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